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Fifth New Drug Seminar: Adriamycin

Division of Cancer Treatment
National Cancer Institute

Editor: Stephen K. Carter

December 16-17, 1974

Washington, DC

Adriamycin: New Drug Seminar

Los Angeles County/University of Southern California Cancer Center
and
Baltimore Cancer Research Center
Division of Cancer Treatment
National Cancer Institute*

Editors: N. R. Bachur
R. S. Benjamin
T. C. Hall

January 15-16, 1975
San Francisco, California

*Co-sponsors: Western Cancer Study Group, Fred Hutchinson Cancer Research Center and Division of Continuing Education of University of Washington, Intrascience Foundation, N. E. Los Angeles American Cancer Society, and Cancer Control Agency of British Columbia.

Jeffrey A. Gottlieb, MD, died on July 1, 1975, at the age of 35. At the time of his death he was Head of the Section of Chemotherapy in the Department of Developmental Therapeutics, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, and Executive Secretary, Southwest Oncology Group. He received his training in medical oncology as a Clinical Associate at the National Cancer Institute, Baltimore Cancer Research Center, and as an Advanced Senior Fellow in the Department of Developmental Therapeutics at M.D. Anderson.

During his own long and quietly courageous battle with the disease he worked so intensively to conquer, his contributions to the field of cancer research were innumerable. It is fitting that this issue of *Cancer Chemotherapy Reports* which summarizes the current status of adriamycin therapy be dedicated to his memory since he, probably more than any other individual, deserves credit for the rapid advancement of this drug from its early clinical trial to its current status in the forefront of cancer chemotherapy. Not only did he recognize the tremendous therapeutic potential of what only a few years ago was a new drug, a fact which led to its widespread use at M.D. Anderson and in the Southwest Oncology Group, but more importantly he recognized its therapeutic limitations. While many of us were pleased to have a drug which was effective in patients with otherwise refractory malignant disease such as adult sarcomas, Dr. Gottlieb was not satisfied and strove for still better results with adriamycin combinations, the achievement of which is well documented elsewhere in this issue. This is but one small example of his continued search for improved treatment of patients afflicted with cancer; for Jeffrey A. Gottlieb, superb administrator, eminent physician, and scientist, was above all a great human being whose consideration for the problems of his patients, friends, and colleagues always took precedent over the obvious problem of his own illness. Few people have lived so full a life in so few years.

He is survived by his wife, Marjorie (Midge) and his two children, Elizabeth and Keith, his parents, and a sister. The Jeffrey A. Gottlieb Memorial Fund has been established at the University of Texas System Cancer Center.

Robert S. Benjamin, MD
Robert B. Livingston, MD
Emil J Freireich, MD

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Development of Adriamycin (NSC-123127)[1]

M. Ghione[2,3]

SUMMARY

A review of the main events which led to the discovery, development, and characterization of adriamycin as a potent and useful antitumor drug is presented. Also briefly outlined are some lines of future work connected with a more exhaustive study of the pharmacokinetic and pharmacodynamic properties of the drug. The immunointerfering and cardiotoxic activities of the drug are examined and the evidence that indicates an interplay of these factors in sustaining some of the phenomena of adriamycin administration is discussed.

[Cancer Chemother Rep Part 3, vol 6:83-89, 1975]

During the development phase of a new drug the qualitative and quantitative effects of the new compound in animal model systems are studied to establish the activity range, the mode of action, and the effective and toxic dose levels, and in clinical therapy, the relevant parameters are identified and checked for their soundness. The final goal of the development phase is to establish an optimal therapeutic strategy, and although it is fairly easy to draw a rough theoretic sketch of the activities sequentially involved in this program, it is very hard to predict the interplay of the different factors involved in the development of a previously unknown compound. These considerations explain the highly variable period of time allotted to the different subphases of development.

About 20 years ago Farmitalia Research Laboratories initiated a program designed to study new compounds produced by new strains of microorganisms isolated from samples of soil collected from all over the world. The search for anticancer drugs was one of the principal purposes of this project, and it gave momentum to the work. It is certainly not a coincidence but rather a consequence of the appropriateness of the time that during the same year the National Cancer Institute started their program to achieve the control of cancer by chemical means. We are happy to acknowledge how much the achievements of these programs are to be attributed to the intuition and faith of the two men who started and actively sponsored them: Drs. C. Gordon Zubrod and Aurelio Di Marco.

The Farmitalia program was started with the combined help in the laboratories of young chemists, biologists, and physicians. Dr. F. M. Arcamone and his associates were keenly interested in the fascinating chemical and biochemical problems of natural product biosynthesis and mechanism of action. The biologists (Dr. C. Spalla and his associates), who had worked in the field of agriculture research, had learned how to deal with the vagaries of soil-dwelling micro-organisms. Some physicians had previous experience and a strong motivation towards experimental and clinical chemotherapy. On these terms, and with the cooperation of other specialists, it was possible to start trials directed at isolating new compounds and characterizing and testing them in animals and, if possible, in man.

A great deal of effort was devoted by our research team, as well as by many others all over the world during the 1950s and 1960s, to the study of the so-called secondary metabolites of Streptomycetes. This approach was justified by empirical observations gathered during the 1940s (largely by the work of S. Waksman and co-workers). Compared to other micro-organisms the Streptomycetes seem to be characterized by a fairly large degree of ductility of the genetic code. Therefore, their enzymatic array can scale at an extent unparalleled by other organisms. This peculiarity can be correlated (either as a cause or as an effect) to the ubiquitousness of the Streptomycetes and, therefore, to the variety of substrates they deal with, in opposition to many other micro-organisms which exist in a more or less steady-state ecologic niche.

The number of previously unknown compounds discovered among the secondary metabolites of the Streptomycetes, as well as the ability to genetically manipulate these organisms (even using the rather crude methods available), justified the interest in this research. The Streptomycetes capability of diversifying and exploring widely divergent metabolic pathways gave us the hope to find among their products new compounds able to interfere preferentially in the metabolism of cancer cells. The wide-range screening carried out as a result of this philosophy led our team to the isolation of some new compounds whose chemical structure and mechanism of action

[1]Adriamycin: CAS reg. No. 23214-92-8.

[2]Farmitalia, Milan, Italy.

[3]*Reprint requests to:* M. Ghione, MD, Farmitalia, Via dei Gracchi, 35 Milan, Italy.

have been elucidated (eg, trehalosamine, lucensomycin, distamycin A, etc), and drew our attention to the anthracyclines.

This generic name designates a group of glucosidic antibiotics possessing an anthraquinol-like tetracyclic aglycone, whose chemistry was elucidated by Brockmann (1). The characteristic red color of these compounds facilitates the gathering of the producing colonies. In 1957 a red pigment-producing colony was isolated from a culture of soil from Puglia, a region of southern Italy. The micro-organism from this soil was christened *Streptomyces peucetius* according to the name of one of the old tribes of that area. The antibiotic produced by this strain was named daunomycin (later designated daunorubicin).[4] As soon as the chemical structure of daunorubicin was clarified by Arcamone et al (2-6) and the antitumor activity was demonstrated by Di Marco et al (7), a comparison of the chemical features of the new antibiotic with those of other anthracyclines endowed with similar but less favorable pharmacologic properties led to the conclusion that the investigation of other compounds structurally related to daunorubicin could result in the discovery of a more active antitumor agent (8).

On this basis and while the studies on the antimitotic activity of daunorubicin were being conducted, our microbiologists made many attempts to modify the genetic code of the strain with the intention of altering its metabolic pathways and obtaining new compounds. They took advantage of the high rate of either spontaneous or induced mutation in *S. peucetius* to isolate some variant strains. One of the strains which produced a bright red pigment was studied more closely. The peculiar color of its aerial mycelium (with hues from blue-green to grey-green) led us to call the strain *S. peucetius* var. *caesius* by adopting a latin adjective indicating the color of the sky (coelum).

The strain was cultured in flasks and fermentation tanks and the analysis of its metabolic products revealed the presence of a new compound: 14-hydroxy-daunomycin (adriamycin). The structure and absolute configuration of the compound were established on the basis of the chemical and spectroscopic evidence (9,10) and a partial synthesis of adriamycin from daunorubicin was also performed (11). The new compound was called adriamycin because of the proximity of the parent strain source to the Adriatic Sea and to the ancient town of Andria (whose name was slightly altered for euphonic reasons). The World Health Organization ad hoc Committee, ignoring this background, called it doxorubicin. Its yield was very low (a few milligrams per liter) and the presence of other similar but uninteresting compounds encumbered the extraction procedures.

A first step in the development of adriamycin was achieved by determining the optimal culture conditions. A study of the many roles played by the yeast in the fermentation process was of paramount significance. The yeast growth factor essential for the strain development was identified with the vitamin B_6. It was also observed that yeast as well as some other compounds can block the feed-back inhibition exerted by adriamycin even at fairly low concentrations on the growth and metabolism of the producing strain. The optimal ratio of growth factors, yeast, and other components of the medium was determined in a long series of trials by challenging the properties of a number of substrains selected on the basis of a higher adriamycin production. This work and its chemical counterpart for the extraction, purification, and analysis systems required many years of effort, but eventually a properly controlled pharmaceutic preparation of adriamycin was developed that was suitable for the study of its biologic properties. The study is still in progress and the up-to-date picture of the results achieved, including the experimental and clinical evidence, will be discussed in other papers in this symposium. However, I think it is appropriate to mention some problems that affected the course of adriamycin development as well as some of the future lines of work. The very beginning was certainly not exciting.

The first results of the biologic tests were more or less deceiving because the effect of adriamycin on "in vitro" cell cultures of different types was not higher (or even lower) than that of daunorubicin tested as a comparison product (12,13). In addition, the first biochemical studies carried out on cell-free systems showed that the DNA-binding properties were similar for both antibiotics. Even the acute toxicity of adriamycin (intravenous [iv] LD50 in mice = 20.8 mg/kg) was higher than that of daunorubicin (iv LD50 in mice = 26 mg/kg), but the discouragement of these data was superseded by the definitely superior antitumor activity exerted by adriamycin in vivo at tolerated doses in comparison with daunorubicin.

Di Marco et al (14) confirmed that adriamycin displayed a higher therapeutic index than did daunorubicin on a variety of experimental tumor systems such as Ehrlich ascites carcinoma, ascitic transplantable lymphosarcoma, solid Sarcoma 180, and solid Oberling-Guérin-Guérin carcinoma. Analogous results have been observed in transplantable leukemia originally induced by Gross leukemia virus (12), L1210 leukemia (15), and spontaneous and transplanted mammary carcinoma (16).

At the same time, subacute and chronic toxicity studies in mice, rabbits, and dogs were being carried out as well as teratologic studies in rats and rabbits. The outstanding properties of the compound soon aroused the interest of the pharmacologists.

Pharmacokinetic studies in mice and rats showed that adriamycin is rapidly cleared from plasma, concentrating in the liver, spleen, kidney, heart, and lung, and excreted in the urine and bile (17-19). Later, it was also observed in man that adriamycin is rapidly cleared from plasma and excreted in the urine and bile, being predominantly metabolized in the

[4]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

liver (20-22). The pharmacodynamic effects of adriamycin were also investigated and particular attention was paid to the influence exerted by the antibiotic on the heart function.

Meanwhile, the experimental data were judged adequate and a phase I clinical study was considered justified. In early 1968, the relevant literature and documentation and a supply of the drug were submitted to the Istituto Nazionale per lo Studio e la Cura dei Tumori in Milan. The phase I and II clinical trials (started in mid-1968) and the subsequent work were carried out under the direction of Dr. G. Bonadonna. The clinical trials rapidly demonstrated that the new compound had antitumor activity in man and the development of adriamycin was strongly enhanced by the enthusiasm generated by the first positive results.

New studies were performed in Europe as well as in the United States at the Memorial Sloan-Kettering Cancer Center in New York, NY. Shortly afterwards, in the first half of 1970, the research on adriamycin was broadened under the auspices of the National Cancer Institute (NCI) in a program of international liaison for the purpose of forwarding the assay of potentially useful antineoplastic agents. The NCI's sponsorship is an example of the advantages that can be secured through international cooperation. Adriamycin development was one of the first and most gratifying results of this agreement.

For the last 4 years the cooperative study groups, the cancer centers, and independent investigators have been studying adriamycin in a variety of experimental and clinical conditions. The results have been the subject of many scientific papers and were discussed at two International Symposia (23,24) specifically devoted to adriamycin, and also at the 11th International Cancer Congress (Florence, Italy, Oct 1974).

Documentation of the drug's activity was directly submitted by Farmitalia to the Food and Drug Administration and the new drug application was accepted. Adriamycin, which has been available for a few years in western Europe, is now also on the US market and will be available in Japan in the near future.

It would be erroneous to believe that having reached this goal, complete adriamycin development has been achieved; therefore, delaying or stopping altogether this development would be premature. We may actually be only at the beginning of an unparalleled scientific and practical achievement. To increase the return on the work carried out during the last 20 years amidst so many difficulties it is necessary to plan the future development. This will include enlarged clinical investigations on adriamycin alone and in combination with other anticancer drugs. This latter task is made easier by the amazingly good capability of adriamycin to elicit additive or synergistic effects when combined with many different antitumor compounds. Moreover, adriamycin shall be integrated into curative, adjuvant, or palliative regimens involving surgery and/or radiation therapy.

Basic and clinical research shall eventually be continued to gain a better knowledge of the mechanism of action as well as of the pharmacokinetic and pharmacodynamic properties of this drug. The aim of this research is to increase the clinical usefulness of the compound by diminishing the unwanted side effects. For this purpose particular attention is paid to the phenomena underlining the most relevant occurrences, ie, the immunosuppression and cardiotoxicity.

The work carried out by Isetta et al (25), Della Bruna et al (26), and Casazza et al (27,28) on different model systems shows that adriamycin possesses a definite immunodepressant activity, but at least in the Moloney sarcoma virus-induced tumor in mice the immunodepressant activity of adriamycin is lower than that of daunorubicin. In fact, the spontaneous regression of the tumor, sustained by the host immune reaction, is adversely affected by daunorubicin and not by adriamycin.

The lower degree (or different type) of immunointerference displayed by adriamycin compared with daunorubicin plays a definite role in determining the higher antitumor activity of the former drug. The disappearance of such differences between the two drugs when the comparative trials are carried out in whole-body irradiated animals (29) can be interpreted in favor of this statement, but from a general point of view the incidence of other factors must be considered, eg, the modification of adriamycin kinetics in animals given X-irradiation (30) and/or the existence of interactions between anthracyclines, ionizing radiations, and targets (31-33) occurring in vivo as demonstrated by the start of recall phenomena (34).

This evidence gives priority to the study of the immunointerfering properties of adriamycin and this will be one of the main goals for future development. A priority is also given to the study of adriamycin cardiotoxicity characterized by a delayed and insidious cardiomyopathy with electrocardiographic abnormalities, dyspnea, cyanosis, and in some cases irreversible congestive heart failure. The properties of inducing depression of cardiac function are not peculiar to the anthracycline derivatives and are shared by other drugs including some widely used antibiotics (35). However, the fairly high incidence of such depression, particularly in patients treated with cumulative doses of adriamycin or daunorubicin greater than 500-600 mg/m^2 (36-38), demanded a careful evaluation of the clinical data and the establishment of experimental work in a field where meager previous experience was available.

Research devoted to the study of the pharmacodynamic properties of daunorubicin and adriamycin (39-42), and chiefly the work by Mhatre et al (43), Herman et al (44-47), Arena et al (48), and Bossa et al (49), and the recent achievements by Bertazzoli et al (50,51), Jaenke (52), Young (53), and Necco et al (54), showed that either the antibiotic or some of its metab-

olites can exert a toxic effect on myocardial cells and impair the cardiac function.

The symptomatology, course, and pathogenesis of this toxic syndrome vary according to the dose and schedule of treatment. The ventricular arrhythmias produced by slow iv infusions of large doses of daunorubicin into golden hamsters are probably mediated through an effect on the central sympathetic nervous system (45).

The permanent myocardial damage induced by the drug can be elicited in experimental animals with the administration of a single high dose of adriamycin or with treatment at repeated low doses. The ultrastructural patterns of the cardiomyopathy induced in rabbits by this latter schedule of treatment are very similar to those observed in man, with degeneration of mitochondria, nucleoli, myofibrillae, and eventually the whole cell.

From the kinetic point of view this evidence can be tentatively explained in different ways (55). A rapid review of some of them can help clarify the problem.

It is legitimate to suppose that during long-term treatment adriamycin progressively accumulates inside a deep compartment until toxic levels are attained and a nonreversible lesion results. This interpretation is consistent with the pharmacokinetic properties of adriamycin, the deep compartment being identified with the mitochondria. Good evidence of the preferential antimitochondrial activity of daunorubicin (56,57) and of adriamycin (58) was first based on the observation that the inhibiting activity of these antibiotics on Saccharomyces is higher in nonfermentable (glycerol) than in fermentable (glucose) medium. In daunorubicin, it was shown that a particular fraction of mitochondrial RNA synthesis is primarily affected.

These findings are consistent with the histologic evidence of early and extended mitochondrial lesions in intoxicated animals and man (32, 47, 59, 60). Some other biochemical lesions take place (61), probably correlated with the interference in Ca^{++} transfer (34,44). It is not surprising that the most conspicuous effects of such lesions are demonstrated by the myocardial function because of its strict dependence upon the mitochondrial function and Ca^{++} balance. If this hypothesis is correct, the cardiotoxic effect can be avoided by preventing drug accumulation. This goal can be reached by prolonging the interval between two subsequent administrations. However, the clinical and experimental evidence achieved so far cannot rule out the alternative interpretation that with every dose of adriamycin, the instantaneous flooding of the tissues due to the high drug concentration (ie, a "bolus" effect) starts a slowly progressing but irreversible lesion of a certain aliquot of the mitochondrial population dwelling inside the cells.

Eventually (and by repeating the flooding), the actual number of mitochondria per cell progressively decreases and eventually it will be so low that the cell will die due to a kind of energetic crisis. If this assumption is correct, the amount of adriamycin per dose should be modified to avoid the high peaks of blood and tissue levels.

These hypotheses can now be submitted to experimental testing and we can also attempt to determine the role played in counteracting cardiotoxic phenomena by cardenolides, according to the experimental data of Arena et al (42) and Necco et al (62), and the clinical evidence offered by Gilladoga et al (63).

Some kind of cross-fertilization occurred between the two main lines of research outlined above (ie, interference in the immune and myocardial function), and generated a new (but not unexpected and keenly sought-after) development area based on the evidence recently gathered by our team on the role played by autoimmune reactions in the pathogenesis of adriamycin-induced cardiomyopathies. According to Fioretti et al (64), the spleen cells of rabbits repeatedly treated with adriamycin exert a strong cytotoxic effect against rabbit embryonal heart cells (REHC), while the control rabbit spleen cells are devoid of any activity. Moreover, in rabbits treated with adriamycin, the indirect immunofluorescence test showed the presence of circulating antibodies which bind to REHC (with different localizations depending on whether or not the cells are pretreated with adriamycin). These antiheart antibodies are fairly specific and are not cytotoxic, even in the presence of the complement, but their binding to REHC not only strongly enhances the cytotoxicity of the adriamycin-treated rabbit spleen cells, but also mediates a cytotoxic attack by control rabbit spleen cells otherwise harmless.

The production of a certain amount of lymphokynes was observed during the course of the cell-mediated cytotoxicity. It is highly probable that the well-known phenomena of recruiting and the direct cytotoxic effect of lymphokynes can contribute to increasing the response. This kind of allogeneic heart cell attack by lymphocytes is not peculiar to adriamycin-treated animals. An analogous phenomenon has been observed in rabbits infected with *Trypanosoma cruzi* and is assumed to be a relevant cofactor in the pathogenesis of Chagas' myocarditis (65). A humoral reaction with antiheart immunofluorescent antibodies appears in rats even after isoproterenol-induced myocardial necrosis (66). These findings are just examples of the role the immune reactions can play in the pathogenesis of many cardiovascular diseases (67), but the problem is a general one and deserves a more careful examination.

Immune reactions are induced by the presence of an antigen(s), and the appearance of new metabolic or cell membrane-associated antigens can be elicited by many factors. Infectious agents play an important role, not only in the budding viruses, eg, myxoviruses (68) or rabies (69), but probably in other intracellular pathogens such as Schizomycetes (including Mycoplasma [70]) and even Protozoa.

It may be of some interest to remember that the action exerted by Mycoplasma can be ascribed to the chromosome changes sustained by this agent (71),

and it deserves particular attention because Mycoplasma as ubiquitous contaminants of cell cultures (72) can induce the appearance of a common antigen(s). In some instances (perhaps in Chlamydia infections [73]) the cell antigenic modifications could play a role in determining and maintaining the chronic lymphocytic reaction characteristic of the granulomatous exudation (74).

Antigenic changes of target cells can follow an antibody wave and elude its efficacy (eg, in *Trypanosoma brucei* infections), but much more interesting from the point of view of tumor chemotherapy are the cell antigen changes induced by drug treatment in vitro (75) or in vivo (76), because they give way, at least in some tumor strains, to the appearance of new strong antigens, which persist after transplantation (77-80) and elicit a cell-mediated reaction (79,81).

Outside the tumor field it is well known that certain drugs such as hydralazine, procainamide, isoniazid, chlorpromazine, trimethodiazine, α-methyldopa, nitrofurantoine, and sulfonamides are connected with drug-induced autoimmune diseases. The drug-induced immune diseases require the existence of new drug-induced antigens, but the mechanisms underlying the start and continuation of these antigens are not yet fully explained and are perhaps not unique.

The DNA-complexing properties of some drugs (chiefly hydralazine, more frequently associated with lupus erythematosus-like disease) have been correlated with the start of antinuclear antibodies (82,83); however, other mechanisms can be involved, such as metabolizing capabilities, photochemical reactions, presence of other chemically reactive compounds, etc (84).

A drug could act by exerting a selective pressure, or by inducing (derepressing) adaptive (perhaps drug metabolizing) enzymes, or through a mechanism of miscoding (85), but it cannot be disregarded that many different compounds (75) can firmly bind to the cell and enhance the immunogenicity. At least one (the guinea pig complement) can be passively carried through the multiplication cycles to the cell progeny to which it gives, even if progressively diluted, a sensitivity towards anticomplement antibodies which lasts for several generations (86).

Certainly, in the next several years, immunologists and pharmacologists will work together and the study of adriamycin can offer a fascinating model. One of the first aspects to be raised in the future development of the drug will be to prove whether adriamycin-induced immune reactions are limited to the heart or also involve other organs and tumors (87). Some hints are given by the observation of a delayed syndrome characterized by autoimmune-like progressive thickening of the glomerular basal membrane in rats treated with a high single dose of daunorubicin but not adriamycin (88).

The balance existing between the immunodepressant and immunoinducing properties of adriamycin shall be carefully examined on the basis of the work by Glynn et al (89) and Mihich (90) on the concurrent and sequential effects displayed on tumors by drug activity and immune reactions. Special attention shall be paid to the role played by these apparently contrasting properties of the drug in delaying the start of cytotoxic effects (either beneficial or adverse) in reference to the period in which the treatment with adriamycin has been carried out.

This shift makes it difficult to evaluate a cause-effect relationship and complicates the already complex task of the experimental physicians. In this context particular attention should also be paid to the design of protocols of combined treatment with adriamycin and other drugs which can exert an immunointerfering activity.

These huge fields of investigation do not exhaust the potential of adriamycin. New lines are shown by the fundamental research (91-93) on the inhibiting activity exerted by adriamycin upon the reverse transcriptase, the viral enzyme characteristic of the oncogenic RNA virus. This work expands the possibility of interfering in a very early stage of oncogenesis, and, together with the research on the activity of adriamycin against DNA virus multiplication (94), represents another new frontier of the adriamycin development.

Finally, we must remember the work by Trouet et al (95,96) and Atassi et al (97) which, according to the principles of the lysosomotropic therapy outlined by De Duve et al (98), explores a new way of treatment by administering a complex of DNA and adriamycin in which the active compound is carried inside the cell towards the target.

To complete the picture we also must mention the chemical work going on in our laboratory as well as in many other laboratories with the object of synthesizing new adriamycin derivatives. These studies (99) will undoubtedly help clarify some problems and elicit many others. We hope that, urged by the convergence of so many intriguing problems, we will be able to move steadily into this fascinating research.

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Adriamycin (NSC-123127): Mode and Mechanism of Action[1]

A. Di Marco[2,3]

After demonstrating the activity of the antibiotic daunorubicin (1-3) (fig 1) (isolated from cultures of *Streptomyces peucetius*) against some experimental tumors and demonstrating the anthracycline structure (4-6), an effort was made to obtain biosynthetic analogs of this compound. This resulted in the product adriamycin (fig 1), which Arcamone et al (7-9) demonstrated to be the 14-hydroxyl derivative of daunorubicin.

In mice transplanted with Ehrlich ascites tumor, adriamycin induced an increased survival time in comparison to daunorubicin (10), and the same effect was observed on transplanted L1210 leukemia (11). Of major importance was the observation of an increased therapeutic index (1.21:0.67; LD10/ID90) in the solid tumor Sarcoma 180, and the substantial activity in a range of transplantable, spontaneous, and virus-induced tumors (table 1).

From the collected data some important observations can be made:

a. If the antibiotic is administered intraperitoneally (ip) to the tumor cells implanted in the intraperitoneal cavity, the tumor may be eradicated with a minimum risk to the host. Because adriamycin also has a considerable systemic effect by this route, the tumor cells are much more sensitive to the effect of the drug than are the affected normal tissues.
b. When the drug is administered systemically the effect on the tumor is obtained with doses which also affect normal tissues; however, in this case it is possible to observe a substantial increase in lifespan of the treated neoplastic animals.
c. A favorable effect is present in the rapidly proliferating experimental leukemias as well as in the slow-growing carcinomas such as mammary cancer in mice.

High doses of daunorubicin given to Moloney sarcoma virus (MSV)-induced tumors (12) cause a transient inhibition of tumor growth followed by a marked recurrence of tumors which continue until death. On the other hand, complete and permanent tumor regression is observed in untreated controls (figs 2 and 3). Tumor recurrence is rare after daunorubicin treatment in mice 20 days old at the time of infection and is absent after treatment in mice 28 days old at the time of infection. Tumor recurrence can be attributed to the action of daunorubicin on the induction and multiplication of immunocompetent cells that react with the virus or virus-induced antigens in tumor cells.

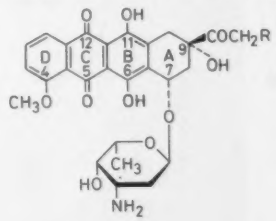
The higher inhibition of tumor growth by adriamycin may be related to the higher activity of this drug in destroying the primary tumor cells. This has been demonstrated in an independent experimental system. However, the different effects of the two antibiotics on the tumor recurrence should be related to a lower impairment by adriamycin of the defense immune mechanism. The effects of both antibiotics are the same on an MSV-derived cell line which lost the ability to produce MSV and was not found to be immunogenic by concomitant immunity experiments (13).

The effect of adriamycin on the production of antiviral antibodies and on the proliferation of the antibody-producing cells is comparable to that of daunorubicin, while the effect of adriamycin on cell-mediated immunity seems to be significantly reduced in comparison to daunorubicin (14). The explanation for these different effects on different cellular districts related to the immunologic response against tumors must be considered in relationship to the peculiar pharmacokinetics of the drugs and to the different proliferative response of antibody-producing cells in comparison to the T lymphocytes.

Pharmacologic factors such as reduced metabolism and the extended persistence in the animal body may be important in explaining the improved activity of adriamycin in comparison to daunorubicin. However, the reasons for the increase in selectivity should be investigated at the cellular level.

Activity on Normal and Neoplastic Mammalian Cells In Vitro

By evaluating the number of mitotic figures (mitotic index), or the proliferative activity of the cell, it was shown that daunorubicin has a strong inhibiting effect on the in vitro growth of a number of cell strains. Adriamycin also has a comparable effect on



daunorubicin : R=H
adriamycin : R=OH

FIGURE 1.—Structures of adriamycin and daunorubicin.

[1]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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TABLE 1.—Activity of daunorubicin and adriamycin on experimental tumors*

Type	Tumor	Activity† of— Daunorubicin	Adriamycin
Ascitic tumors	L1210 leukemia	+	++
	P388 leukemia	++	+++
	P288 leukemia	+	++
	Ehrlich carcinoma	+	++
	Sarcoma 180	+	++
	Hepatoma MH-134	+	++
	Yoshida sarcoma	++	++
Disseminated leukemias	Murine leukemia virus, transplanted	++	++
	Gross leukemia virus, transplanted	+	+
Solid tumors	Sarcoma 180	+	++
	Mammary carcinoma	+	+++
	Lewis lung carcinoma	±	+
	MSV (murine) sarcoma	±	+
	Lymphosarcoma	+	+
	Oberling-Guérin-Guérin myeloma	+	±

*From ref Nos. 10,11,42-46.
†± = None; + = minimal; ++ = moderate; +++ = significant.

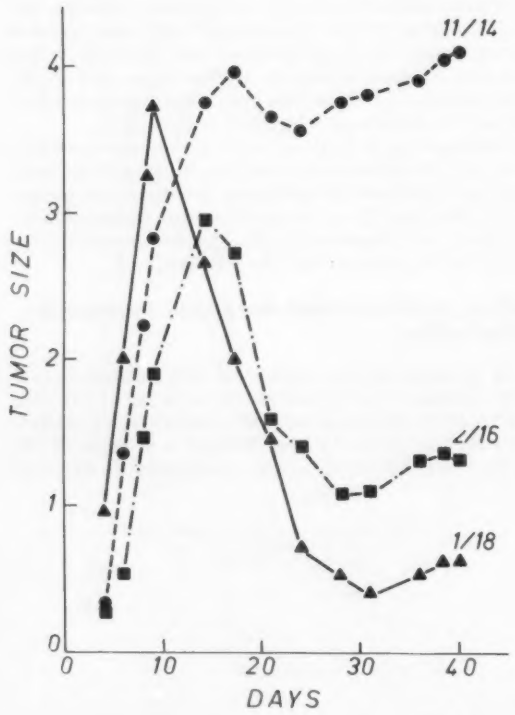

FIGURE 2.—Effect of pretreatment with daunorubicin and adriamycin on MSV-induced tumors in 16-day-old mice. Treatment by subcutaneous route, at Days 3, 2, and 1 before the infection. ▲ = Controls; ● = daunorubicin (7.5 mg/kg/day); ■ = adriamycin (5.2 mg/kg/day). [*From* ref 12.]

many of these cell strains (table 2). Cell damage induced by daunorubicin is primarily nuclear, ie, in resting cells there is a finely granular appearance of the chromatin and a marked alteration in the shape and size of the nucleoli, while cytoplasmic changes, such as vacuolization, are moderate and appear only after prolonged treatment with high doses. The damage to the nucleolar structure appears quickly after the addition of daunorubicin to the medium and

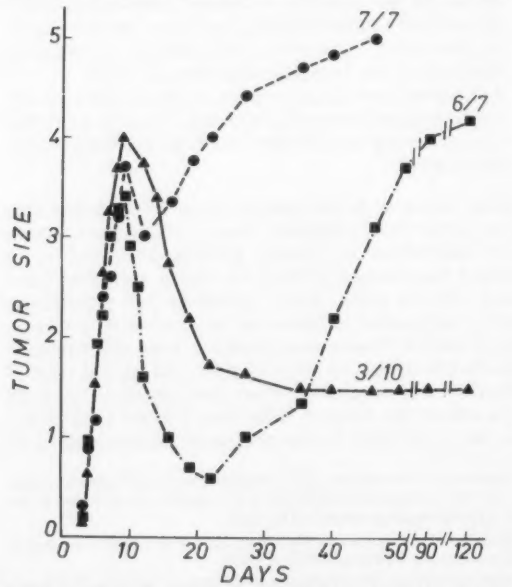

FIGURE 3.—Effect of daunorubicin and adriamycin on MSV-induced tumors in 16-day-old mice. Treatment from Days 4 to 11 after the infection. ▲ = Controls; ● = daunorubicin (3.25 mg/kg/day); ■ = adriamycin (2.25 mg/kg/day). [*From* ref 12.]

TABLE 2.—Effect of daunorubicin and adriamycin on viability of different cell lines*

	ID50 (μg/ml) at various times after treatment					
	Daunorubicin			Adriamycin		
Cell lines	2 hrs	8 hrs	24 hrs	2 hrs	8 hrs	24 hrs
HeLa	0.098	0.036	<0.025	0.33	0.19	<0.025
3T3	Inactive	0.09	0.4	Inactive	ND†	0.09
3T12	1.0	0.09	0.082	0.9	ND	0.04
SVT 2	Inactive	0.073	0.068	Inactive	ND	0.08
MS-2 (46th passage)	0.08	0.04	0.04	0.09	0.04	0.03
MS-T (25th passage)	0.09	0.08	<0.01	0.07	0.09	0.07

*From ref No. 47.
†ND = no data.

can be followed by electron microscopy. After 2 hours of incubation HeLa cells, treated with 1 μg/ml of daunorubicin in the medium show disorganization and fragmentation of the nucleolar filamentous structure with detachment of the RNA granules. After 24 hours of treatment the nucleolus appears as a very compact structure with the appearance of vacuoles. The injuries to the nucleolar structure should be considered to be related to the inhibition caused by daunorubicin of ribosomal RNA synthesis.

During mitosis, chromosomal damage such as fragmentations and mitotic aberrations (primarily anaphasic bridges) have been described (3) (figs 4 and 5). If a sufficient amount of daunorubicin is added to rat fibroblast cultures, it is possible to observe, by phase contrast microscopy, an immediate block of the mitotic process and an anomalous scattering of the chromosomes within the cell.

Mode of Action

The fluorescence of daunorubicin shows the penetration of this substance into the cell and the binding to nuclei (fig 6). The radiochemical data confirm that the largest amount of daunorubicin that enters the cells is taken up by the nucleus (fig 7). However, some labeling is also found in mitochondrial and microsomal fractions (Rusconi A, unpublished observations). After extraction according to Crampton, it was shown that nearly all the antibiotics can be found in the nucleoprotein fraction extracted with 2 M NaCl (15). Experiments with ^{3}H-daunorubicin confirm that after a short period of in vitro incubation,the largest part of daunorubicin is bound to the nucleoprotein fraction.

The uptake of daunorubicin by HeLa cells is strictly dependent upon the concentration in the medium (16). Results from experiments on serum-

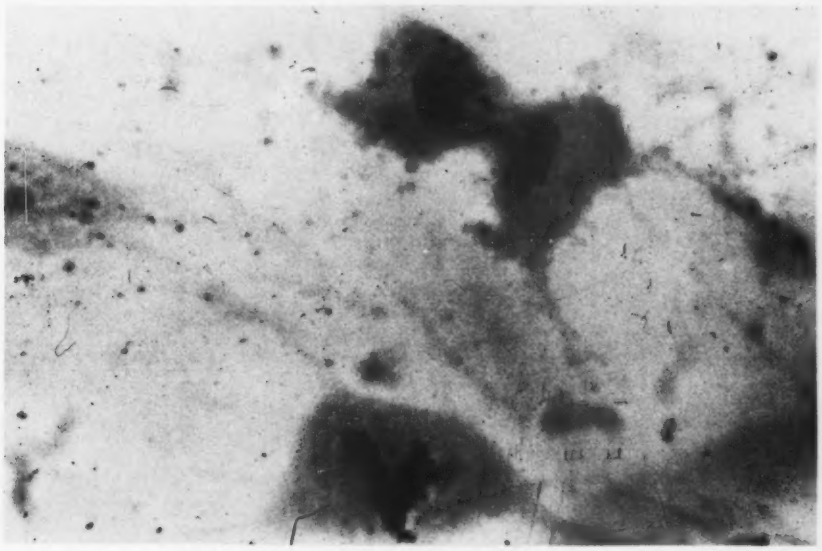

FIGURE 4.—Chromosomal damage.

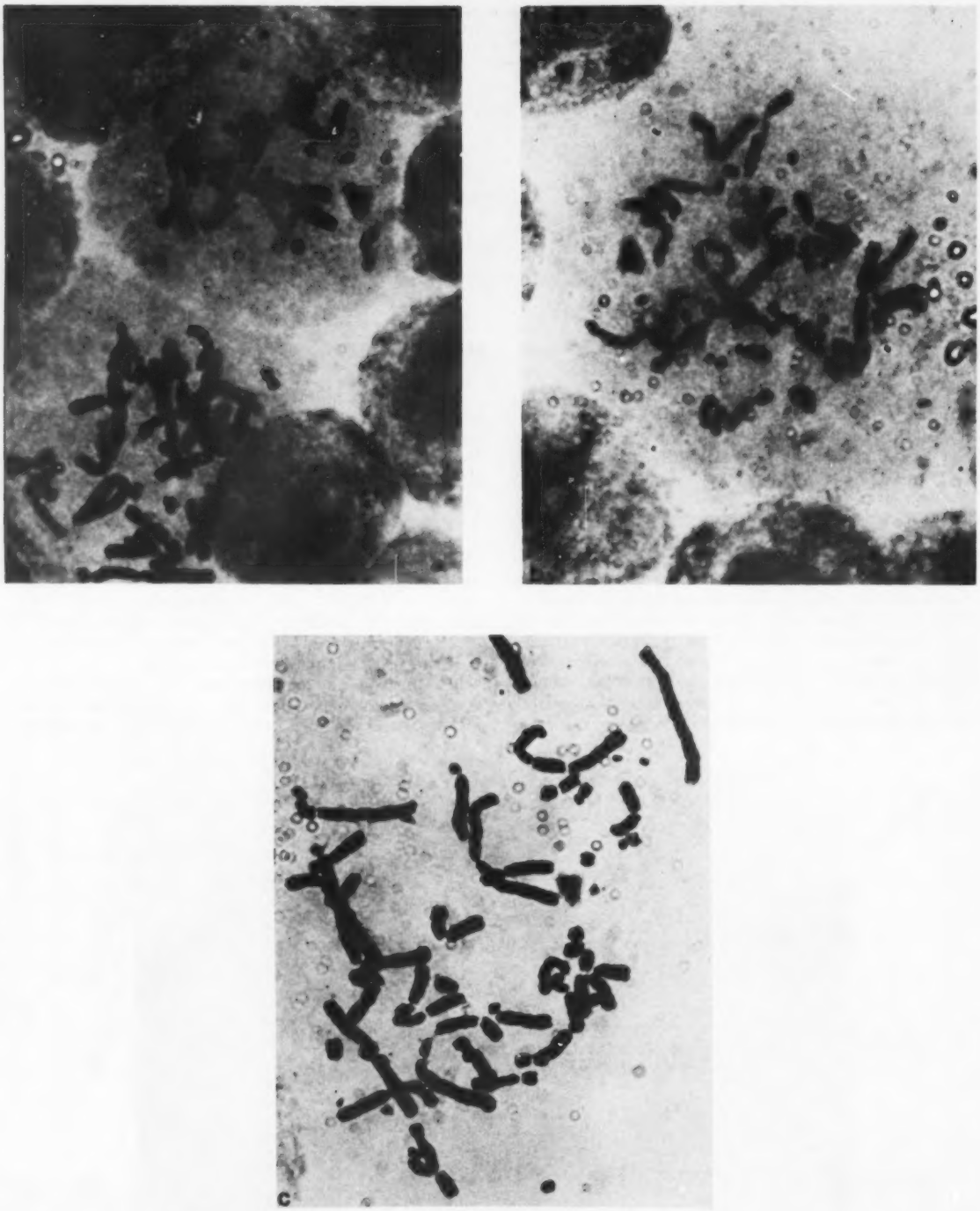

FIGURE 5.—Chromosomal damage during mitosis (a,b,c).

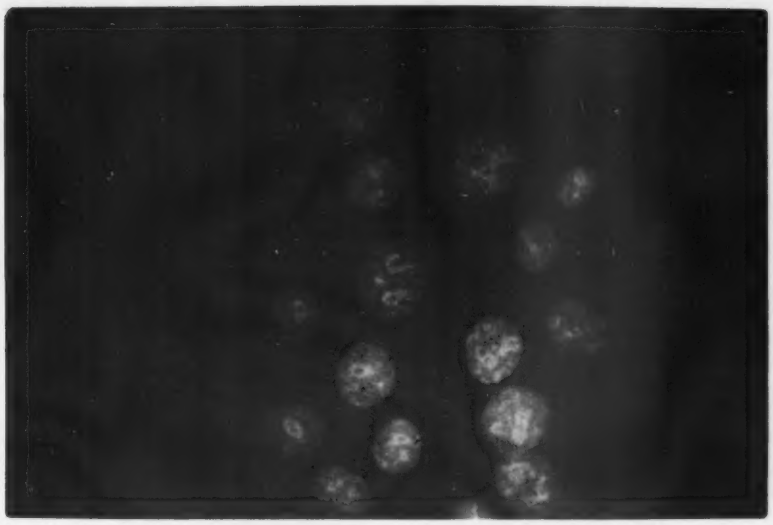

FIGURE 6.—Daunorubicin entering the cells and being taken up by the nucleus. [*From* ref 17.]

FIGURE 7.—Uptake of ^{3}H-daunorubicin in synchronized cultures of rat fibroblasts. [*From* ref 19.]

stimulated cells show that resting cells may also take up the drug from the medium (Supino R, Boretti G, and Di Marco A, unpublished data). In synchronized cultures of rat fibroblasts it was observed (17) that the uptake of ^{3}H-daunorubicin occurs throughout the regenerative cycle, but reaches its maximum during DNA duplication as revealed by the ^{3}H-thymidine uptake curve (fig 8).

The main biochemical effect of the anthracyclines is concerned with nucleic acid synthesis. The binding to DNA is believed to be responsible for its interference with the template DNA function in transcription and duplication processes (18). Daunorubicin forms a strong complex with DNA which is associated with the manifold physicochemical changes presented in table 3. Three types of interaction are responsible for the stabilization of the complex; the first is the electrostatic interaction between the protonated amino group of the sugar residue and the ionized phosphate groups. This is proved by the reduced complexing ability of *N*-acetyl-daunomycin. The changes in the response after modification of the sugar moiety suggest steric requirements, as for example either the distance between the intercalating site and the phosphate anion or the orientation of the amino group. A second type of interaction is due to hydrogen bonds, as shown by the effects displayed on the complex by substances such as formamide and urea. The third interaction is represented by the intercalating processes, which are sustained by the weak hydrophobic stabilization, taking place between the intercalating molecule and the adjacent base pairs in close contact (and by the diminished repulsion between the negatively charged phosphate groups as a result of the local increase of the distances between the base pairs).

TABLE 3.—Physicochemical changes observed after formation of the complex daunorubicin-DNA

1. Hypochromic and bathochromic visible changes in the antibiotic.
2. Reduction of antibiotic fluorescence (Calendi et al, 1965).
3. Increase in the melting temperature of the DNA (Kersten and Kersten, 1965; Zunino et al, 1972).
4. Decrease in the buoyant density of the DNA (Kersten et al, 1966).
5. Increase in the intrinsic viscosity of the DNA (Zunino, 1971).
6. Decrease of sedimentation coefficient of the DNA (Calendi et al, 1965; Kersten et al, 1966).
7. Protection of DNA from degradation by nucleotic enzymes (Kersten and Kersten, 1968).

The conclusions mentioned above are confirmed by the roentgenographic studies of Pigram et al (19) on DNA fibers (for which the drug: DNA-P molar ratio ranged from 0.03 to 0.2). The diffraction pattern indicated that the complex had a conformation very similar to that of B-DNA, and the intercalation model was found to account for the diffraction data and was stereochemically satisfactory (fig 9).

Pigram et al found that the amino sugar moiety of daunorubicin is in the large groove of DNA, and the hydrophobic faces of the base pairs and the drug overlap extensively. The ionized amino group interacts with the second DNA phosphate anion away from the intercalation site, and an interaction (hydrogen binding) between the C-9 hydroxyl of daunorubicin and the first phosphate is also possible.

The equilibrium measurements derived from spectrophotometric analysis and equilibrium dialysis (20) and the viscosity measurements are consistent

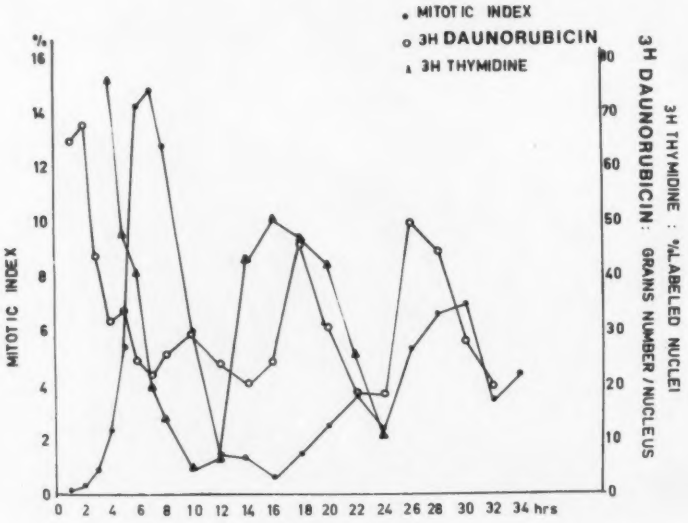

FIGURE 8.—Uptake of ^{3}H-daunorubicin in synchronized cultures of rat fibroblasts. [*From* ref 27.]

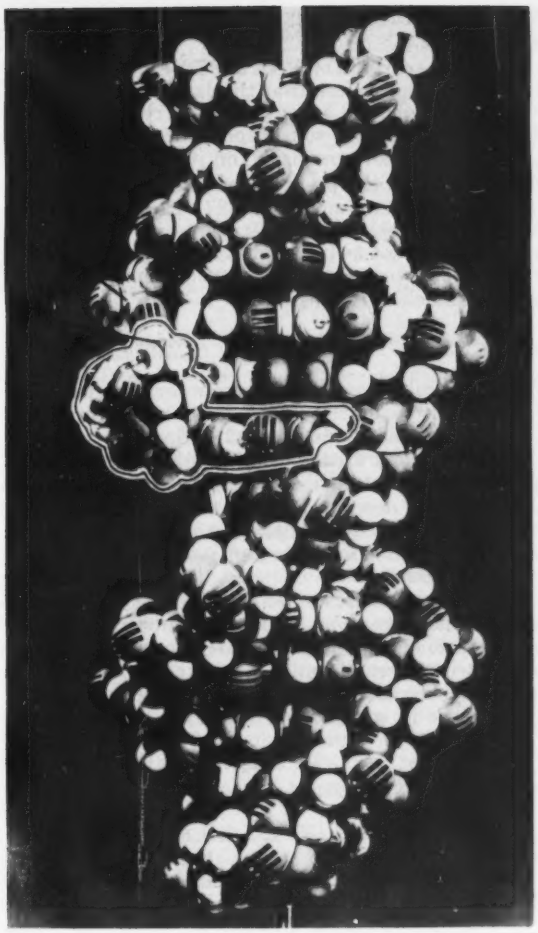

FIGURE 9.—Intercalation of daunorubicin into DNA double helix.
[*From* ref 19.]

with the intercalation model of Pigram. Also consistent with this model are experiments in which daunorubicin was found to cause the uncoiling of the supercoiled closed-circular DNA in the same qualitative fashion as other drugs believed to intercalate (21,22).

It is still not clear whether the binding ability of daunorubicin to DNA is the same for every kind of DNA or whether there is a preferential binding to some regions of DNA (depending on a specific base sequence or on some peculiar feature of the DNA's structure). Daunorubicin displayed little base composition specificity as determined by the decrease in buoyant density of different native DNA's in a CsCl gradient (23) (or by the degree of inhibition of DNA-directed RNA polymerase reaction using different DNA primers) (24). It has also been reported, on the basis of circular dichroism spectra, that complex formation with DNA is only slightly dependent on the G-C content of DNA (25). However, the stabilization of the double helix by daunorubicin appears to be dependent on the A-T content in the DNA (fig 10).

The remarkable effect observed on DNA and RNA synthesis may be related to the in vitro-observed inhibiting activity on DNA and RNA polymerase reactions (table 4, fig 11). The activity of some enzymes related to DNA synthesis, such as thymidine kinase and deoxycytidine monophosphate deaminase, increases and DNA polymerase is not reduced after treatment of HeLa cells with daunorubicin. This indicates that the drug does not act on the formation of the last enzyme but, as pointed out by Kim et al (26), acts on the unavailability of the template to the enzyme as a result of the interaction with DNA.

In a study of the RNA synthesis reaction catalyzed by *Escherichia coli* RNA polymerase (27), it was observed that the daunorubicin-induced inhibition may be overcome by an increase in the concentration of DNA. However, it is unaffected by an increase in the concentration of the enzyme, which is consistent with an inactivation of the template function of DNA in the polymerase reaction. The inhibiting effect of the anthracyclines on the template activity of DNA could be based on the prevention of

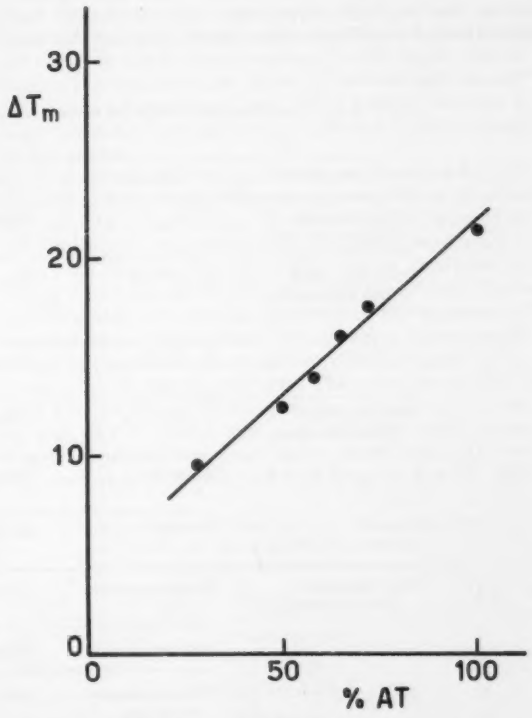

FIGURE 10.—Relation of the stabilization of the double helix by daunorubicin to the A-T content in the DNA.

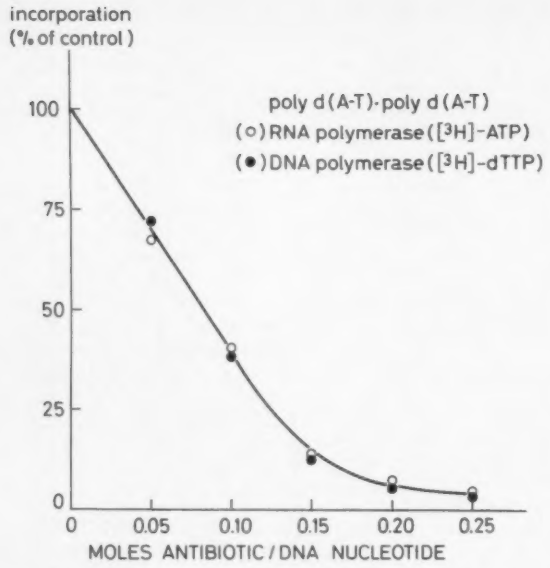

FIGURE 11.—Inhibiting activity observed in vitro on DNA and RNA polymerase reactions.

the local denaturation of the helix that takes place during the replication process. Alternatively, the distortion of the DNA structure caused by the anthracyclines could inhibit the binding of the polymerase to DNA.

From experiments on RNA and DNA polymerase in vitro, the results show that the daunorubicin inhibition of the template activity of DNA replication and transcription shows some dependence on DNA composition, and more precisely, on its contents in A-T base pairs and the presence of alternating A-T sequences on the same strands (27). However, the results of the inhibition experiments on polymerase reactions cannot be immediately extrapolated to the in vivo-observed inhibition of nucleic acid synthesis which is much more sensitive:

$$\frac{\text{Daunorubicin}}{\text{DNA nucleotides}} = \frac{1}{60\text{–}100}$$

for 50% inhibition of uridine incorporation in HeLa cells (28) in comparison to a ratio of 1:5 for RNA polymerase reaction in vitro (27). One may suppose that the enzymes used for the inhibition experiments are not those working in the in vivo synthesis, and in the situation described above the complex of the enzyme with the DNA template present in chromatin is working with much higher efficiency and sensitivity to inhibitors.

To what extent are the inhibiting effects on nucleic acid synthesis responsible for the inhibition of cell proliferation? An antimitotic effect has been observed with doses of daunorubicin which do not affect the macromolecular synthesis and in the cell-cycle phase in which a nucleic acid synthesis may

TABLE 4.—Studies on the effect of daunorubicin and adriamycin on DNA and RNA polymerases

Enzyme	Source	Inhibitor	Template	Ref No.
DNA-dependent DNA polymerase	*E. coli*	Daunorubicin	Crab's, DNA, dAT, dG-dC (dT. dC)	50,24
DNA-dependent RNA polymerase	*E. coli* K12	Daunorubicin	Thymus DNA; poly d(A-T)·poly d(A-T)	51
	E. coli K12	Daunorubicin	Poly dG·poly dC; *M. lysodeikticus* DNA	27
DNA-dependent DNA polymerase	*E. coli* B	Daunorubicin	Activated thymus DNA	50,51
	E. coli B	Daunorubicin	Calf thymus DNA; *M. lysodeikticus* DNA	52
	E. coli B	Daunorubicin	Poly [d(A-T)]; poly dG·poly dC	27
RNA-dependent DNA polymerase	Murine leukemia virus	Adriamycin	Endogenous RNA	53
	MSV (murine)	Daunorubicin	Endogenous RNA	35
	Friend leukemia virus and Rous sarcoma virus	Adriamycin	Poly dA-dT; poly rA(dT)2; poly (dT·dC)	54

TABLE 5.—Inhibition of DNA-polymerase activity of MSV (murine) by daunorubicin derivatives in the presence of various templates*

Antibiotic*	^{3}H-thymidine monophosphate incorporation into DNA poly (dA-dT) (cpm/reaction mixture)	^{3}H-deoxyguanosine-monophosphate incorporation into DNA poly (dI-dC) (cpm/reaction mixture)	^{3}H-thymidine monophosphate incorporation into poly (rA) · oligo $(dT)_{12}$ (cpm/reaction mixture)
None	5446 (100)	3757 (100)	1196 (100)
Daunorubicin	126 (2.3)	4123 (109)	467 (39)
Adriamycin	106 (1.9)	4252 (113)	472 (39.4)
13-Dihydro-daunomycin	162 (3)	6446 (171.5)	552 (46.1)
N-guanidine-acetamide daunomycin	781 (14.3)	4897 (130.3)	572 (47.8)
N-acetyl-daunomycin	5607 (102.9)	4086 (108.7)	1160 (97.0)

*Antibiotic concentration = 5 μg/reaction mixture (0.25 ml). The figures in parentheses indicate the percent of control. [*From* ref 35.]

not be perceived. Furthermore, a compound such as *N*-acetyl-daunomycin, which has a poor effect on nucleic acid synthesis, is able to stop the mitotic activity (29,30).

The mechanism of the antimitotic effect of daunorubicin and adriamycin is quite obscure but it conceivably may involve spindle fiber function. The cross resistance observed between daunorubicin and adriamycin and the vinca alkaloids may point to a common antimitotic mechanism of action as well as to an aspecific reduction of the drug uptake.

Studies on synchronized cell cultures (30,31) show that an antiproliferative activity may be obtained by exposing the cells to daunorubicin during different phases of the cell cycle. However, cell killing is more pronounced when cells are exposed during the S phase (26).

The data of Kim and Kim (26) show that the exposure of HeLa cells for a short period to an adriamycin dose that causes a limited reduction in ^{3}H-thymidine uptake is enough to give rise to an extensive cell death. Therefore, the cell killing may be the result of selective damage to the chromosomal DNA molecules rather than quantitative reduction in the amount of DNA synthesized. The effect of daunorubicin on DNA synthesis in synchronously replicating cells (rat fibroblasts) is higher during late S phase (32), when heterochromatic portions of the chromosome are normally replicated (ie, nucleolus-associated chromatin and centromeres). The binding of the antibiotic to these regions may be facilitated by the presence of repetitions of A-T rich homopolymer-like DNA sequences. This observation may help us to understand the pre-eminent effect on the nucleolar organization, the function, and the chromosomal structure. The extensive chromosomal damages observed after daunorubicin and adriamycin treatment of cells in culture (33) may be related to the observation of foci of transformed cells in vitro in cultured mouse embryonic cells (Necco A, et al, unpublished results). On the other hand, an interference with mitochondrial DNA expression and duplication could be the basis of the cardiotoxic effects, which until recently were poorly understood.

Similarly, when added to the incubation medium at different times before and after infection, daunorubicin and adriamycin, as well as the other inhibitors of DNA or DNA-dependent RNA synthesis, inhibit the "foci" formation induced by MSV on mouse embryo cells (34) and in parallel, the replication of MSV (Casazza AM, and Soranzo C, unpublished results).

A similar effect was reported for actinomycin D on the replication of Rous sarcoma virus and indicated the presence of a DNA step in the virus multiplication. Therefore, the recent finding that daunorubicin and adriamycin inhibit the reverse transcriptase activity of RNA tumor viruses is of major importance. The inhibition exerted by both drugs against the DNA polymerase from RNA tumor viruses is also dependent on the type of primer-template used in the assay.

As shown in table 5 the reactions directed by poly d(A-T) and, to a lesser degree, the reactions directed by poly(rA)-oligo$(dT)_{12}$, are sensitive to daunorubicin inhibition. In contrast, in the presence of poly dI · poly dC, the enzyme from MSV is not inhibited by 20 μg/ml of daunorubicin (or its derivatives). Table 5 also shows the results obtained in the presence of daunorubicin derivatives. Under the same conditions, daunorubicin and adriamycin are most effective (table 6). The higher sensitivity to daunorubicin and adriamycin inhibition of the reaction directed by poly d(A-T) co-polymer, also with the viral polymerase, confirms the previously mentioned data on the preferential binding of this drug to d(A-T) · d(A-

TABLE 6.—Effect of adriamycin and daunorubicin on cellular and viral DNA polymerases

Enzyme	Drug	ID50 (μM)
MSV polymerase	Daunorubicin	15
MSV polymerase	Adriamycin	11
Rat liver polymerase	Daunorubicin	38
Rat liver polymerase	Adriamycin	36

**From* ref No. 55.

T) base pairs. 13-Dihydro-daunomycin behaved similarly to daunorubicin, but *N*-acetyl-daunomycin was completely inactive (35). These results indicate that the inhibition exerted by daunorubicin and adriamycin on DNA polymerase from RNA tumor viruses is selectively dependent on the structure of the primer templates used in the assay system. Further, the inhibiting activity requires specific structural features similar to those observed for the formation of a stable complex with DNA.

There may be a question raised as to the possible importance of this effect for the therapeutic activity in the special case of experimental virus-induced tumors such as MSV(T) and some leukemias. The observed reduction in the tumor's growth may be the result of different processes such as: (*a*) inhibition of proliferation of virus-transformed cells; and (*b*) inhibition of the virus-multiplication in the cells and consequent reduction in recruitment of transformed cells. The experimental evidence available in this system to distinguish between these two effects is scanty; however, in another related system, such as the splenomegaly induced by the Chirigos MSV plasma variant, it is possible to observe a reduction in virus content in the plasma at a time when there is not yet a noticeable increase in spleen weight. The reduction in virus production, obtained directly by selective inhibition of the virus-replicating system or indirectly by selective killing of virus-infected cells, may play an important role in the therapeutic activity in these experimental tumors. The possible meaning for the therapeutic activity in man should be studied in Burkitt's lymphoma.

The theory that the biologic effects of these antibiotics are strictly related to the formation of a stable complex with DNA is strengthened by the observation that in a series of daunorubicin analogs there is a strict relationship between the inhibiting effect on nucleic acid synthesis in vitro, inhibition of proliferative activity, and DNA binding ability as measured by physicochemical methods (table 7). In HeLa cells the effect of adriamycin on DNA and RNA synthesis is reduced in comparison to daunorubicin, in agreement with the lower antiproliferative effect. This adriamycin activity is apparently not related to a different uptake in these cells as shown in figures 12 and 13.

The possible selective effect of daunorubicin and adriamycin against tumor cells may be sought in comparable cell lines such as BALB/c 3T3, the corresponding in vitro transformed SVT_2, and the spontaneously transformed 3T12. Both antibiotics considerably reduce the efficiency of the transformed cells in comparison to the original 3T3 line. In the 3T12 cells the daunorubicin uptake is nearly twice that of adriamycin; therefore, the killing effect of intracellular adriamycin is higher in comparison to daunorubicin (fig 14). The lower uptake of adriamycin is confirmed in a different experimental system (ie, the conditioned mice embryonic fibroblasts) in which DNA synthesis is stimulated by serum. A comparable inhibition of DNA synthesis has been observed in this system with both antibiotics, with an intracellular concentration of adriamycin that is from one half to one fourth that of daunorubicin (table 8).

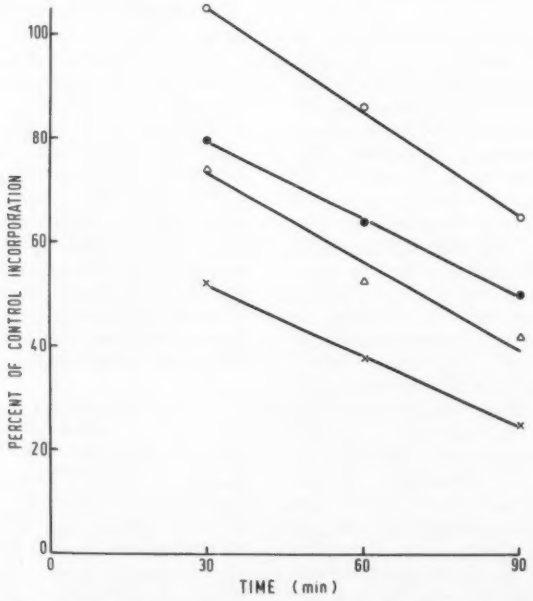

FIGURE 12.—Effect of daunorubicin and adriamycin on DNA and RNA synthesis directed by calf thymus DNA. ●, ○ = Daunorubicin; ▲, △ = adriamycin.

TABLE 7.—Biologic effects and DNA binding ability of daunorubicin derivatives

Antibiotics	R[B]‡	C-13	Amino sugar	K_{APP}* (M^{-1})	ΔTM, calf thymus DNA*	ID50 (μg/ml), DNA synthesis	ID50 (μg/ml), RNA synthesis	ID50 (μM), cell proliferation†
Daunorubicin	H	O	Daunosamine	2.9×10^6	13.4	0.04	0.1	0.89
Adriamycin	OH	O		2.8×10^6	14.8	0.06	0.5	1.9
Daunosaminyl-daunomycin	H	O	4-Daunosaminyl-daunosamine	—	—	—	—	5.5
Daunomycin-thio-semicarbazone	H	$NNHCSNH_2$	Daunosamine	—	—	—	—	8.3
13-Dihydro-daunomycin	H	HOH	Daunosamine	1.1×10^6	9.8	0.09	0.2	7.9
N-Guanidine-acetamide-dauno-mycin	H	O	*N*-Guanidine-acetamide-daunosamine	7.5×10^5	8.9	1.6	2.2	7.6
2-Amino-2-deoxy-glucosyldauno-mycinone			2-Amino-2-x-D-deoxy-glu-cose	7.1×10^4	8.0	—	—	8.8
N-Acetyl-daunomycin	H	O	*N*-Acetyl-daunosamine	1.8×10^4	1.0	2.5	3.4	—

*From ref No. 20.
†From ref No. 56.
‡See fig 1.

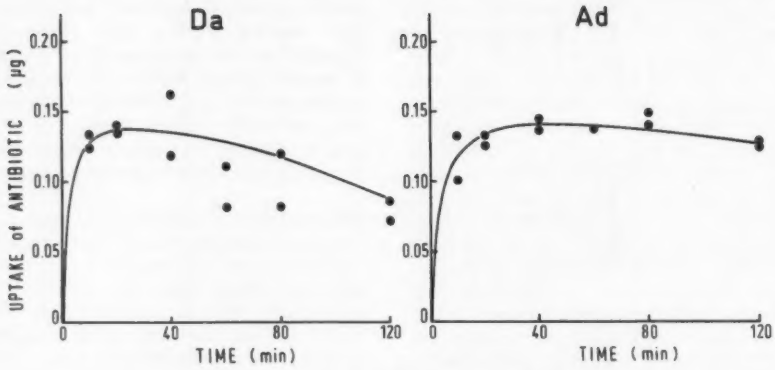

FIGURE 13.—Inhibition of the "foci" formation induced by MSV on mouse embryo cells and of cell proliferation.

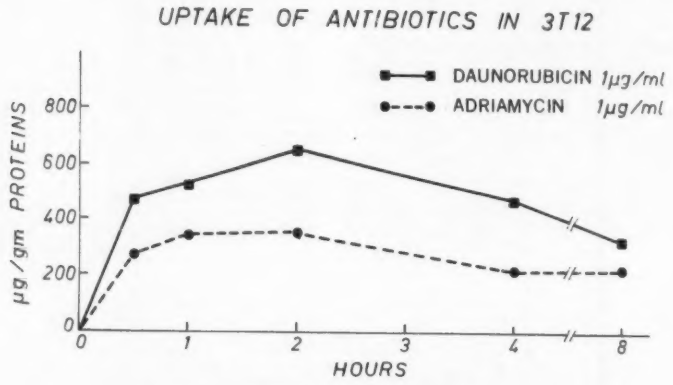

FIGURE 14.—Uptake of antibiotics in the 3T12 cell line.

These results, which are in agreement with previous data of Meriwether and Bachur (36) on L1210 cells, indicate that the ability of cells to take up adriamycin is considerably lower than that for daunorubicin; however, when in the cell, adriamycin is more active. In fact, as previously shown, the inhibiting effect on DNA polymerase reactions of adriamycin in vitro is higher than that of daunorubicin (table 9). Since the association constant to DNA is nearly the same for the two compounds, the greater effect of adriamycin could be tentatively related to a reduced speed of dissociation.

TABLE 8.—Daunorubicin and adriamycin uptake in contact-inhibited and FCS-stimulated mice embryonic fibroblasts*

Culture condition	^{3}H-thymidine incorporation in DNA (cpm/ μg DNA)	Drug uptake in cells	
		Daunorubicin (μg/6 × 10^6 cells)	Adriamycin (μg/6 × 10^6 cells)
Contact-inhibited	1.19	1.47	0.34
FCS-stimulated	35.0	1.33	0.38

*Drug concentration was 2 μg/ml medium, ie, 20 μg/plate. FCS = fetal calf serum.

TABLE 9.—Inhibiting effect on DNA polymerase reaction in vitro

Enzyme	ID50 concentrations	
	Daunorubicin	Adriamycin
M. lysodeikticus DNA polymerase	1.6×10^{-5}M	1.2×10^{-5}M
E. coli RNA polymerase	1×10^{-5}M	0.6×10^{-5}M

The effect of the *S. peucetius*-derived antibiotics and their semisynthetic analogs on different experimental tumors may also be related to the binding ability to DNA. The lower activity displayed by 13-dihydro-daunomycin is only partly understandable on the basis of the lower stability of its DNA complex and the significantly lower inhibition of DNA and RNA metabolism when compared to daunorubicin. In agreement with these binding parameters is the very low activity of the *N*-acetyl derivative. The other derivatives with low binding capacity had little or no activity.

The effects of the semisynthetic analogs as well as the effects of a semisynthetic glycoside of daunorubicin are shown in table 10. These derivatives, although still retaining appreciable in vitro activity, are practically inactive in the animal tests.

Derivatives of daunorubicin at both the amino and the methylketone function have also been prepared and tested at the Stanford Research Institute. In the first class of derivatives, with the possible exception of *N*-acetyl-daunomycin, all compounds were inactive, in agreement with our findings of the strict requirement for the ionizable amino group in the sugar residue. The derivatives at the side chain carbonyl retained significant levels of activity on L1210 leukemia in mice (37). French authors have described encouraging clinical results in acute leukemias with daunomycin-benzoylhydrazone (38), which could not be extended to solid tumors (39).

Table 11 shows the effect of adriamycin esters on some experimental tumors. The activity displayed by these compounds is of the same order of magnitude as that of the parent compound (40). For the octanoate derivative, it was possible to demonstrate the conversion to adriamycin by serum esterases. If the biologic activity is a consequence of this phenomenon, then the more favorable results obtained in vivo in comparison to adriamycin could be ascribed to a different distribution in the animal tissues of the parent antibiotic when administered in the form of octanoic acid ester.

It seems likely, from the available knowledge, that the biologic effects of tetracycline antibiotics are related to the binding ability to DNA. However, the

TABLE 10.—Activity of daunorubicin derivatives on a spectrum of experimental tumors

Antibiotic	Activity at optimal dose					
			Sarcoma 180 ascites		Gross leukemia	
	Sarcoma 180 solid*	MSV (murine) sarcoma†	Average survival time (days)‡	Long-term survivors§	Average survival time (days)‡	Long-term survivors§
Daunorubicin	50	63	222	6/11	176	0/10
Adriamycin	31	54	227	8/11	187	1/30
Daunosaminyl-daunomycin	53	88	195	4/10		
13-Dihydro-daunomycin	59	58	231	2/10	153	0/10
Daunomycin-semi-carbazone	100	100				
Daunomycin-thio-semicarbazone			103	0/10		
Daunomycin-oxime		100	130	0/10		
N-guanidine-acetamide-daunomycin			100	0/10		
2-Amino-2-deoxy-glucosyldauno-mycinone			107	0/10		

*Tumor growth = % T/C tumor weight index at Day 11.
†Tumor growth = % T/C ratio at maximal tumor volume (Day 10 after tumor inoculation).
‡Control = 100.
§No. of survivors/No. treated.

TABLE 11.—Activity of optimal doses of adriamycin

Derivative	Ref No.	Sarcoma 180 solid*		MSV (murine) sarcoma†	
		Dose (mg/kg/day)	Tumor growth (%) compared to controls	Dose (mg/kg/day)	Tumor growth (%) compared to controls
Controls			100		100
Daunorubicin	15	3.25	50	3.25	63
Adriamycin	15	2.5	31	2.25	52
Adriamycin-14-acetate	15	2.5	36	1.5	45
Adriamycin-14-propionate	15,25	3.25	33		
Adriamycin-14-octanoate	23	3.5	27	2.75	10
Adriamycin-14-benzoate	15,23,48	3.25	32		
Adriamycin-14-(phenylacetate)	15,23	3.25	38		
Adriamycin-14-nicotinate	49,25	3.25	36		

* Treatment ip on Days 1-8.
† Treatment iv or sc on Days 3-5.
‡ Treatment ip on Day 1.
§ Treatment iv on Days 3-5.
¶ Treatment q2d iv on Days 1-11, 17-27.
** 60 days from tumor transplantation.
†† Dissolved in 5% aqueous ethanol.

specificity of the binding to DNA of different composition and structure of adriamycin seems to be limited. To design chemical derivatives with a higher degree of selective anticancer activity would require a deeper understanding at the molecular level of the malignant transformation of the cells and the ability to increase the affinity of the drugs for some genes in the transformed cells.

LIST OF COMPOUNDS

N-acetyl-daunomycin: NSC-118714; L-*lyxo*-hexopyranoside, 3β-acetyl-1, 2, 3, 4, 6, 11-hexahydro-3, 5, 12-trihydroxy-10-methoxy-6, 11-dioxo-1α-naphthacenyl 3-acetamido-2. 3, 6-trideoxy-, α-, compd. with isopropyl alcohol (1:1)
Actinomycin D: NSC-3053
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
Adriamycin-14-acetate: NSC-149581; CAS reg. No. 41962-27-0; adriamycin, 14-acetate, hydrochloride
Adriamycin-14-benzoate: NSC-149585; CAS reg. No. 41962-28-1; adriamycin, 14-benzoate, hydrochloride
Adriamycin-14-nicotinate: NSC-149583; adriamycin, 14-nicotinate, monohydrochloride
Adriamycin-14-octanoate: NSC-149584; adriamycin, 14-octanoate, hydrochloride
Adriamycin-14-(phenylacetate): NSC-149582; CAS reg. No. 41962-30-5; adriamycin, 14-(phenylacetate), hydrochloride
Adriamycin-14-propionate: NSC-149586; CAS reg. No. 41962-29-2; adriamycin, 14-propionate, hydrochloride
2-Amino-2-deoxy-glucosyl-daunomycinone
Daunomycin-benzoylhydrazone: NSC-164011; CAS reg. No. 36508-71-1
Daunomycin-oxime: NSC-143491; CAS reg. No. 34610-60-1
Daunomycin-semicarbazone: NSC-143114; CAS reg. No. 38942-70-0; daunomycin, 3-semicarbazone, monohydrochloride, dihydrate
Daunomycin-thiosemicarbazone: NSC-165976; hydrazinecarbothioamide, 2-[1-[4-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-1, 2, 3, 4, 6, 11-hexahydro-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-2-naphthacenyl]ethylidene]-, stereoisomer, monohydrochloride, dihydrate
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6; daunomycin
Daunosaminyl-daunomycin: NSC-140781
13-Dihydro-daunomycin: NSC-180510; CAS reg. No. 26164-47-6; 5,12-naphthacenedione, 10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(1-hydroxyethyl)-1-methoxy-, (8*S-cis*)-, hydrochloride
***N*-guanidine-acetamide-daunomycin**

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derivatives on a spectrum of experimental tumors in mice

Sarcoma 180 ascites‡			Gross leukemia§			Mammary carcinoma¶	
Dose (mg/kg/day)	Average survival time	Long-term survivors**	Dose (mg/kg/day)	Average survival time	Long-term survivors**	Dose (mg/kg/day)	Tumor incidence (%) compared to controls
	100	0/10		100	0/10		100
0.25	222	6/11	3.25	176	0/10		
0.5	227	8/11	2	187	1/30	2	50
2	252	7/10	2.5	168	0/10	2.5	10
2	232	1/10	4	147	0/10		
2	250	5/10	4.1 ††	214	3/30	2.5	20
1	287	2/10	2.5	138	0/10		
4	207	2/10	3.25	156	0/10		
2	250	1/10	4	227	0/10		

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Cell Interactions: Determinants of Selective Toxicity of Adriamycin (NSC-123127) and Daunorubicin (NSC-82151)[1,2]

Herbert S. Schwartz and P. M. Kanter[3,4,5]

SUMMARY

Presently, the major determinants of the selective cytotoxicity of adriamycin and daunorubicin appear to reside in their mechanisms of action, their biotransformations, and their uptake and retention by various cells and organs. As an example of selective uptake, lymphocytes from patients with acute myelocytic leukemia and other leukemias accumulate daunorubicin preferentially at levels two to five times greater than adriamycin. In contrast, both agents are taken up to about the same extent by experimental tumor cells (eg, L1210 and P288) although initial rates of uptake appear to be different. In treated mice, daunorubicin accumulates to a greater extent than does adriamycin in the spleen. This differential uptake by a lymphoid organ in the host suggests that daunorubicin may be the more immunosuppressive agent and that the greater therapeutic efficacy of adriamycin may depend upon host participation to some extent. Thus, in mice pretreated with immunosuppressive doses of whole-body X-irradiation (250 R) or subjected to a splenectomy prior to P288 implantation, the subsequent therapeutic effectiveness of adriamycin is reduced considerably. In contrast, the efficacy of daunorubicin, which is considerably less than that of adriamycin in control animals, is essentially unaffected by the pretreatment. The effectiveness of daunorubicin may be increased, however, if spleen cells from sensitized donors are administered as adjuvant therapy. The modes of action of the anthracycline antitumor agents may also contribute to their selective cytotoxicity. Extensive DNA damage in tumor cells may be observed after therapeutic doses (2.5-10 mg/kg) of adriamycin and daunorubicin are administered to mice bearing lymphocytic leukemia (P288). When P288 is incubated with daunorubicin for 2 hours, there is a dose-dependent increase in DNA damage with increasing drug concentrations. Under conditions which produce minimal DNA damage, cell growth and thymidine incorporation are not inhibited by daunorubicin ($1\text{-}5 \times 10^{-8}$ M). Higher concentrations ($25\text{-}125 \times 10^{-8}$ M) which cause extensive DNA fragmentation inhibit both incorporation and growth. Adriamycin also shows evidence of inducing progressive DNA damage after cells are incubated for 2 hours with the drug.

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Within the 5-year span since its discovery, adriamycin has become one of our most potent weapons against solid neoplasms. The question is whether or not this new drug has unique properties which endow it with a selective action—an antitumor specificity that is so rarely found among chemotherapeutic agents. Clearly it falls within a class of DNA-reactive drugs that is remarkable both for its specificity of binding and for its diverse biologic actions (1). Present information suggests that there are two generalized modes of reversible binding to DNA: (*a*) intercalative, in which the reactive ligand is permitted to slip into the helical structure between the stacked bases, and (*b*) adlineation, in which the agent binds primarily to the outside of the DNA, usually by ionic forces. The intercalative drugs (eg, adriamycin and daunorubicin) generally possess multiple planar aromatic ring systems that permit the agents to move into hydrophobic regions between bases. Examples of the latter are usually strongly charged molecules and the most noteworthy include polyamines (eg, spermine and spermidine, etc), the antineoplastic DDUG, and the antibiotics chromomycin A_3 and mithramycin.

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[2]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

[3]Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Memorial Institute, Buffalo, NY.

[4]The authors express gratitude to E. J. Kelly and S. Sipos for their conscientious technical assistance, Dr. J. J. Wang for use of unpublished data, Dr. S. Gailani for providing access to human cells, Dr. E. J. Modest (Sidney Farber Cancer Center) for providing unpublished information, and especially Dr. James Holland (Mt. Sinai School of Medicine) for his interest and encouragement.

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Adriamycin and daunorubicin apparently bind by both modes which probably accounts for their strong affinity for duplex DNA.

In this class of DNA-reactive drugs must also be included a number of alkylating agents which react covalently with DNA. Mitomycin C is the classic example; its binding properties apparently depend upon the enzymatic reduction of the quinone ring to a semiquinone for orientation within the DNA structure and for activation of the aziridine ring (2-4). Recently it has been shown by Kohn and Glaubiger et al (5,6) that the reaction of anthramycin with DNA is irreversible after acid-catalyzed rearrangement. Since the reaction is covalent and requires guanine, the binding to double-stranded DNA blocks the attachment of actinomycin D at the same site.

While detailed information concerning the precise nature of the binding of adriamycin and daunorubicin will undoubtedly contribute to our understanding of their selectivity, other factors must, of necessity, contribute as well to their actions at a target cell. Figure 1 summarizes in a simple scheme the main parameters that may contribute to their specificity. The principal determinants of cytotoxic selectivity are drug uptake (reaction 1), metabolism by enzymes to inactive or to active products, and binding and mechanisms of cytotoxicity which may include DNA breaks and repair (reactions 6 and 7, respectively). The model does not take into account such diverse factors as host-tumor relationships, although these will be discussed briefly as related to differences in drug uptake and antitumor efficacy by the drugs in transplanted leukemias.

SELECTIVITY AND DRUG UPTAKE

In a number of comparative studies, it has been shown that leukemic cells take up daunorubicin more rapidly and to a greater extent than adriamycin (table 1). In human leukemic cells there is no apparent lag in uptake of either adriamycin or daunorubicin, although the initial rate is somewhat slower for adriamycin, with both drugs reaching a plateau by 30-60 minutes. In an earlier study with leukemia L1210 in DBA/2 mice it was shown that there was an initial delay of about 30 minutes in the uptake of adriamycin. The drugs reach similar concentrations at 1 hour and then fall together. Meriwether and Bachur (7) demonstrated that despite differences between the two agents in their initial uptake in vitro, adriamycin appears to be equally as effective as daunorubicin with respect to inhibition of incorporation of labeled precursors into RNA and DNA. Using L1210-treated mice, Wang et al (8) also demonstrated similar inhibitory effectiveness for the two agents. Clearly though, adriamycin is the superior agent in prolonging the survival of hybrid mice (C57Bl × DBA/2 F_1) bearing leukemia L1210 (9).

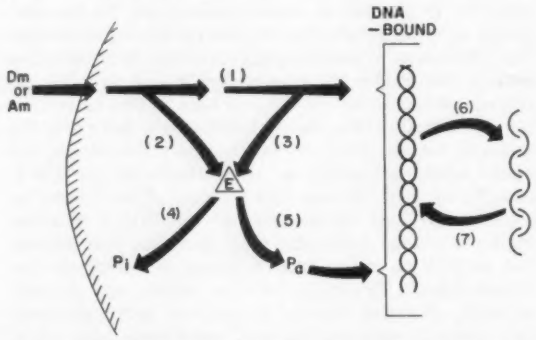

FIGURE 1.—Principal parameters influencing cytotoxic selectivity: drug uptake (1), metabolism by enzymes (E) to inactive (Pi) or active (Pa) products of adriamycin and daunorubicin.

HOST PARTICIPATION

Our studies have been carried out with the lymphocytic leukemia P288 which was syngeneic in DBA/2 mice. Administered intraperitoneally (ip), 100 cells are lethal to 97% of the mice. In this system, a single dose of adriamycin increases the survival time two to four fold (with 20%-30% long-term survivors) whereas daunorubicin can effect no more than a 40% increase and no long-term survivors (10). Yet the uptake of the drugs by P288 is similar, and the uptake fails to explain therapeutic differences. The differences in therapeutic response to the two agents can be completely eradicated, however, by prior treatment of the mice with a low dose of whole-body irradiation (250 R) administered 24 hours before tumor implantation (fig 2). Both irradiated and control mice respond to daunorubicin with a 40% increase in survival time. In contrast, the effect of adriamycin is reduced by host irradiation to the same level as that seen after daunorubicin treatment. The results suggest that, after immunosuppressive doses of irradiation, the efficacy of the drugs is identical with respect to the survival time of tumor-bearing DBA/2 mice.

Some recent results strongly support the idea that the spleen may play a cooperative role in producing long-term effects with adriamycin. As shown in figure 3, splenectomized mice have the same median survival times as the sham-operated mice when treated with daunorubicin at 1.25 mg/kg (ip × 1). This same dose of adriamycin doubles the survival times of sham-operated animals with six of 15 mice surviving for more than 25 days, whereas splenectomy reduces the therapeutic response of adriamycin-treated mice almost to the level obtained with daunorubicin. These results are strikingly similar to those obtained with irradiation.

ADJUVANT EFFECTS OF LYMPHOID CELLS

We have attempted to use lymphoid cells from various organs from syngeneic donors as adjuvant therapy in DBA/2 mice bearing leukemia P288 and treated with daunorubicin. In preliminary experiments, spleen and bone marrow cells from normal donors

had no effect on the response of P288-bearing mice to daunorubicin (1.25-5.0 mg/kg). In a second series, the donors were sensitized by a single injection of lethally irradiated P288 cells (1×10^6, ip, irradiated with 2500-3000 R). Spleen cells from these donors, sensitized for varying periods of time, are capable of increasing the daunorubicin response in test mice from about 16 (25+%) to 19 days (48+%) as shown in table 2. The effect is lost when the sensitization time of the donors is increased to 30 days and the dose of daunorubicin is increased to 5 mg/kg, or when cells from bone marrow or thymus (not shown) are used.

Taken together, the results of the studies with whole-body irradiated mice, splenectomized mice, and those treated with adjuvant spleen cells from sensitized donors all tend to support the idea that the activity of adriamycin against transplanted tumors may depend in part upon an active participation by the host. Thus, selective differences between adriamycin and daunorubicin, insofar as the host response is concerned, may be due to the greater cytotoxicity by daunorubicin toward some population of lymphoid cells, perhaps most notably in the spleen. This is supported in the findings by Yesair et al (11) and by us (10) that daunorubicin is taken up rapidly and accumulated to levels at least twice those of adriamycin by the spleen in normal and in tumor-bearing mice.

CELLULAR RESISTANCE

There are other factors in addition to the uptake of drugs that may also participate in their selective cytotoxicity. Thus, an agent must be retained in an active form (or one that is at least potentially active) at an effective concentration for a long enough time to express its cytotoxic modality. From this it follows that a more rapid uptake or a higher intracellular level of daunorubicin, for example, in lymphoid cells is not a guarantee that this agent will be more cytotoxic than adriamycin. As noted by Meriwether and Bachur (7), the rate of metabolism of daunorubicin to inactive forms may be more rapid in some animal tissues and species than that of adriamycin. As a consequence, adriamycin may be present at effective concentrations longer than daunorubicin, even though the latter may be present initially at higher levels. Altered binding of the agent to macromolecular sites and altered intracellular distributions may also be factors in the selectivity in some cells. Danø et al (12) showed for example that there is a reduced inhibition by daunorubicin of the synthesis of ribosomal (45S) RNA in resistant Ehrlich ascites tumor cells, whereas the synthesis and processing of other RNA forms are not affected to the same degree. Furthermore, the strongly diminished inhibition could not be explained by the relatively small reduction in drug uptake by the resistant cells. Indeed, Wang and his colleagues (8) showed that L1210 cells, made resistant to either daunorubicin or adriamycin, were cross-resistant to adriamycin. Daunorubicin uptake was reduced in the daunorubicin-resistant lines, but uptake of adriamycin by the adriamycin-resistant L1210 was not different from that of the sensitive lines.

MYOCARDIAL TOXICITY

Host toxicity by the anthracycline antitumor agents is an important concern not only for the immunosuppressive effects but also for the life-threatening cardiac toxicity that places a limit on the total dose that may be administered to an individual. Efforts to limit such toxicity are presently developing in numerous laboratories throughout the world, but these efforts have been hampered until quite recently by the lack of a sensitive animal model. Last year, an important step in this direction was taken when it was observed by Jaenke (13) and by Gilladoga et al (14) that rabbits respond to daunorubicin and adriamycin with a delayed cardiomyopathy similar to that observed in humans. Development of this model may prove essential to our understanding of myocardial toxicity and our ability ultimately to prevent or to control it.

TABLE 1.—Uptake of daunorubicin and adriamycin by human lymphoid cells incubated for 60 mins

Cell type	No. of patients	Uptake (ng/10^6 cells) of —		Daunorubicin/ adriamycin ratio
		Daunorubicin	Adriamycin	
Acute lymphocytic leukemia	4*	250 ± 110†	60 ± 53†	4.1 ± 1.6†
Acute myelocytic leukemia	6	465 ± 105†	215 ± 55†	2.2 ± 0.4†
Chronic myelocytic leukemia	1	800	210	3.8
Multiple myeloma (marrow)		510	155	3.3
		670	300	2.2

*Chervinsky DS, and Wang JJ. Unpublished data.

† ± SD.

TABLE 2.—Survival of DBA/2J female mice with P288 after treatment with daunorubicin and cell replacement from sensitized donors*

Daunorubicin (mg/kg)	Day of irradiation of sensitized donors	Adjuvant treatment†— None	To spleen	To marrow
0	1	12.6 ± 2.1	11.9 ± 1.0	12.5 ± 1.5
2.5	1	16.3 ± 2.7	19.0 ± 5.5	16.6 ± 1.6
0	5	13.0 ± 1.4		
2.5	5	16.0 ± 4.3	19.0 ± 2.7	18.0 ± 0.8
0	9‡	12.4 ± 1.8		
2.5	9‡	15.0 ± 0.9	18.3 ± 3.3	16.7 ± 2.3
0	36	12.3 ± 1.3	11.7 ± 0.9	12.7 ± 1.5
2.5	36	17.0 ± 2.0	16.6 ± 1.3	16.7 ± 2.1
0	30	12.3 ± 0.3	13.5 ± 1.8	12.0 ± 0.4
5.0	30	17.0 ± 1.9	14.3 ± 2.1	15.1 ± 1.9

*Donors were sensitized by ip administration of irradiated P288 (2500-3000 R) for various time periods before administration to test mice. Test mice were transplanted with tumor on Day 0 and received 10^6 spleen or marrow cells 24 hrs after treatment with daunorubicin or saline (Day 1).

†Average survival time (days) ± SD.

‡Cell replacement on Day 3.

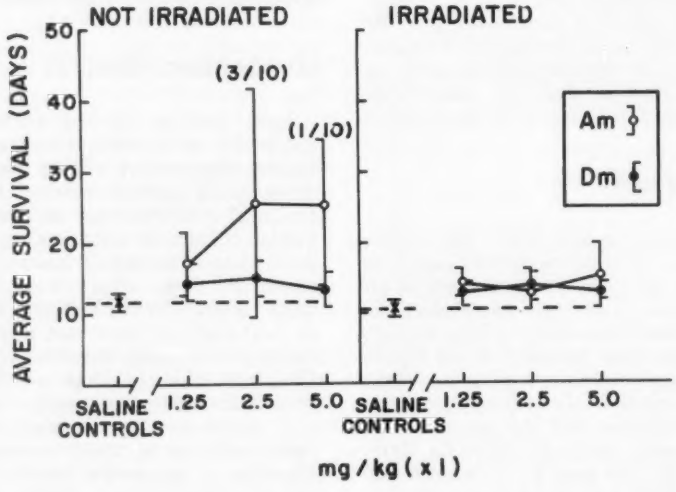

FIGURE 2.—Average survival of DBA/2 mice implanted with P288 ascites tumor (Day 0) and treated on Day 1 with a single ip dose of adriamycin (Am) or daunorubicin (Dm). Mice were irradiated (250 R) or constrained without irradiation on the day before transplantation. [*From* ref 10.]

NEW THERAPEUTIC MODALITIES

Other avenues are presently being explored to alter or to control host toxicity. One of the most promising has been the use of the anthracycline drugs as "lysosomotropic" complexes with DNA. The concept, as developed by Trouet et al (15), requires that a drug be firmly bound to a digestible carrier, as adriamycin or daunorubicin is bound to DNA. Such complexes are expected to be effective only in those cells that possess both high pinocytotic and mitotic activities. Normal cells possessing only one or the other or neither of these attributes should be protected from the agents. The specificity of such agents would be clearly enhanced if the complex is taken up by pinocytosis into tumor cells, if the drug is released within the target cells by lysosomal or other hydrolytic enzymes that digest the carrier, and if the tumor target has both of these qualities in addition to its mitotic activity. In studies with L1210 in DBA/2 mice (16), the complex of adriamycin with herring sperm DNA was half as toxic and about twice as potent as adriamycin alone. Preliminary results (17) with patients having acute myeloblastic

leukemia and acute lymphocytic leukemia also suggest that such complexes may improve the therapeutic index of adriamycin and daunorubicin. These promising endeavors deserve our broadest and most imaginative exploration as soon as possible.

MODE OF INTRACELLULAR ACTIONS

About a year ago (18), we presented preliminary evidence that DNA breaks are found in alkaline sucrose gradient profiles of P288 after the administration of a therapeutic dose of daunorubicin (5 mg/kg, ip × 1). Similarly, extensive damage after treatment with adriamycin occurs in P288 as well. With low doses of either drug (0.62 mg/kg), damage may appear as early as 1 hour after daunorubicin or 3 hours after adriamycin, and it may persist for 1-3 days (19). Clearly, however, such experiments in animals are complicated by host responses to the tumor cells and to the drugs, and it has proved impractical to try to separate an early and possibly primary effect of the drugs from their cytolytic actions in animals. For these reasons, the studies were continued with P288 grown in culture. The cells grow rapidly in primary and continuing suspension culture to a density of 2-5 × 10^6 cells/ml with a doubling time of 14-16 hours in RPMI 1640 medium with 10% fetal calf serum (heat inactivated) and supplemented with glutamine.

After P288 cells were incubated under these conditions for 2 hours with daunorubicin, we found evidence of slight DNA damage (ie, decreased sedimentation velocity) at concentrations of 1-5 × 10^{-8} M and more extensive damage at 2.5 × 10^{-7} to 1.25 × 10^{-6} M (fig 4). Another study (fig 5) with adriamycin shows evidence of progressive damage after cells were treated for 2 hours with high drug concentrations (1.25-2.5 × 10^{-6} M).

In parallel studies the growth of P288 was also measured after 2-hour incubations with daunorubicin. As in the previous study, cells were washed and resuspended in fresh growth media after limited exposure to the drug. Results indicate that lower concentrations (1 and 5 × 10^{-8} M) that produce minimal damage to the DNA do not inhibit growth. At 2.5 × 10^{-7} M, growth is inhibited by about 80% (assayed at 72 hours) and it is completely blocked after higher concentrations. This cytostasis may persist for as long as 7 days after the brief exposure period to daunorubicin.

Thymidine incorporation was also measured in cells exposed to these concentrations of daunorubicin. During the first 2 hours of exposure there is little or no effect with concentrations (1 and 5 × 10^{-8} M)

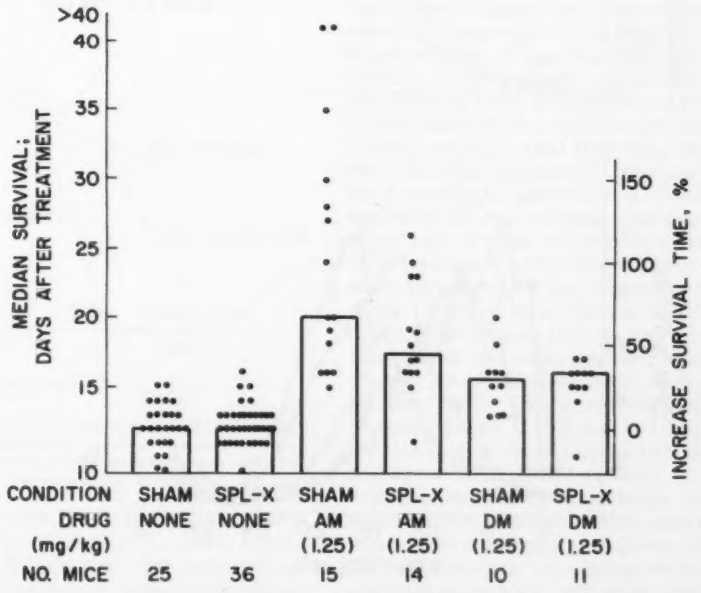

FIGURE 3.—Survival of splenectomized DBA/2J mice bearing P288 ascites tumor and treated with a single ip dose of adriamycin (AM) or daunorubicin (DM). Mice were splenectomized or sham-operated 7 days before transplantation (Day 0) and treated with AM, DM, or saline (ip × 1) on Day 1.

that produce minimal DNA damage but do not inhibit growth. After the higher doses, there is a 40% inhibition of incorporation at 2.5×10^{-7} M and it is almost completely blocked after the first hour at 1.25×10^{-6} M.

Summarizing these studies (fig 6), extensive fragmentation of DNA occurs during a 2-hour incubation with high concentrations of daunorubicin which also inhibit thymidine incorporation and growth. But damage to the DNA is also discernible after the brief exposure of cells to concentrations of daunorubicin as low as 1 and 5×10^{-8} M, concentrations which do not affect either the initial thymidine incorporation or subsequent growth.

That these low concentrations do have biologic effects is shown in the growth of the cells during continuous exposure (fig 7). As indicated here, the presence of even such low concentrations as 1×10^{-8} M of daunorubicin may inhibit growth by 80% when present continuously rather than for only 2 hours. Adriamycin is somewhat less potent during this continuous exposure, requiring about a 50% higher concentration to obtain a similar degree of inhibition, and the relative damage to DNA is also correspondingly less.

CONCLUSION

These recent studies may shed some light on a number of observations by others that have been perplexing us. It seems clear, for example, that agents that bind as strongly to DNA as do adriamycin and daunorubicin might also inhibit polymerase activity (8). As a consequence, the agents should be S-phase specific (20); if they inhibit RNA transcription, a blockade in G_1 would also result. However, Silvestrini et al (21) demonstrated in S180 ascites that neither uridine incorporation into RNA nor thymidine incorporation into DNA was inhibited 90 minutes after the administration of adriamycin and

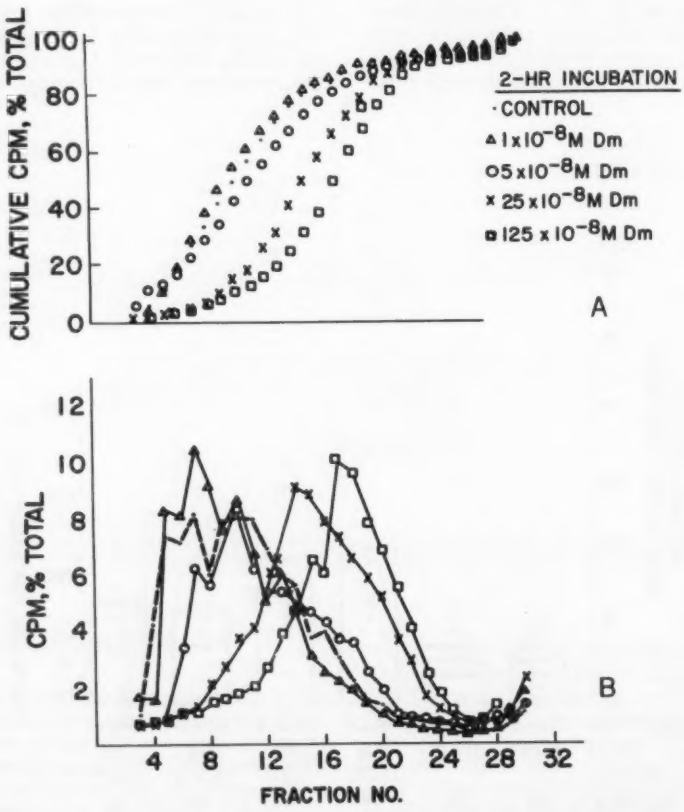

FIGURE 4.—Distribution of DNA (prelabeled with 2-^{14}C-thymidine) from P288 in alkaline sucrose gradients (5%-20%). Daunorubicin (Dm) was incubated with cells for 2 hrs in darkness and the cells were lysed in 0.3 N NaOH overlaying the gradient. Centrifugation was for 60 mins at 30,000 rpm (20°C) in SW 36 rotor. A = cumulative radioactivity in the gradients as % of total recovered. B = radioactivity in each fraction as % of total recovered. Fraction 1 is the bottom of the tubes.

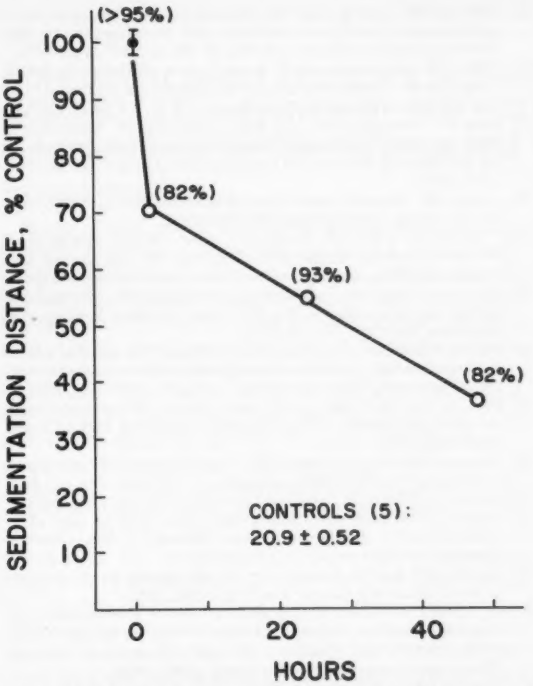

FIGURE 5.—Median sedimentation distance (as calculated from cumulative radioactivity; see fig 4A) of prelabeled DNA from cells incubated for varying times with adriamycin (1.25-2.5 × 10^{-6} M). Figures in parentheses indicate % of cells that exclude trypan blue.

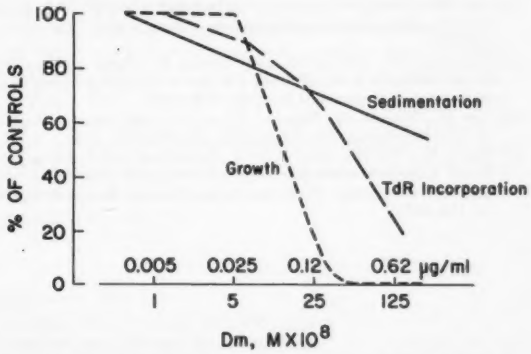

FIGURE 6.—Summary of effects of 2-hr exposure of P288 to varying concentrations of daunorubicin (Dm), as % of change from controls. Curves for sedimentation of DNA were from data from 3-5 experiments and median values estimated as in fig 4A.

daunorubicin. Yet, at this time, the mitotic index was already reduced by 60%-95% by both drugs. Barranco and Novak (22) showed that nondividing Chinese hamster ovary cells were sensitive to adriamycin but not to the phase-specific drugs hydroxyurea and cytosine arabinoside. It was also reported that adriamycin kills cells in G_2 as well as in S

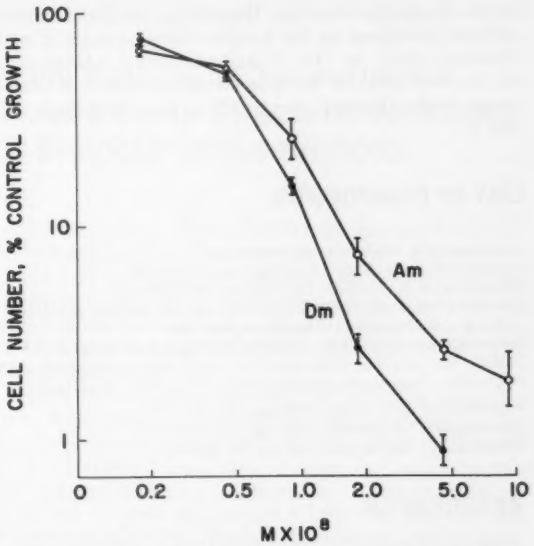

FIGURE 7.—Growth of P288 incubated continuously with daunorubicin (Dm) or adriamycin (Am). Cells were incubated at an initial density of 3-5 × 10^4 cells/ml in a total volume of 21 ml with varying concentrations of Am or Dm. Growth was assayed at 72 hrs. Final density of controls was 6.0-8.4 × 10^5 cells/ml.

phase. Thus phase-specificity of the anthracyclines does not appear to be a prominent consequence of their actions.

It is also noteworthy that both agents cause early and severe chromosomal aberrations (23, 24). In this sense, the observation of Sternberg et al (25) that daunorubicin is carcinogenic in rats is even more difficult to understand unless the drug does more than bind to DNA and inhibit nucleic acid synthesis.

That adriamycin and daunorubicin are not strictly phase-specific, that they may inhibit mitosis without affecting precursor incorporation, that they produce mitotic aberrations, and that they have a potential for carcinogenic changes are observations which may, in time, be related to the production of DNA damage. Furthermore, the prolonged effects of adriamycin and daunorubicin may be due to the additional insult of an inhibition of DNA repair mechanisms by the drugs (26). In any event, it seems prudent to express some concern that delayed cardiac damage may be a response to such prolonged actions by these drugs. This manifestation of toxicity might be exacerbated, if it is due to DNA damage, by combinations with other agents that also produce DNA damage or inhibit DNA repair.

In considering the possibility of additive cardiac toxicity, it is imperative that great caution be exercised when adriamycin or daunorubicin is combined for a therapeutic advantage with irradiation, with alkylating agents, or with other DNA-reactive agents such as actinomycin D. It is for these reasons that the development of animal models assumes such great importance for the investigation of mecha-

nisms of cardiac toxicity. Hopefully, cardiac toxicity will be minimized by the further development of new analogs, such as the *N*-trifluoroacetyl-adriamycin derivative (27), or by the administration of these drugs in the form of selectively active complexes (15, 16).

LIST OF COMPOUNDS

Actinomycin D: NSC-3053; dactinomycin
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
Chromomycin A_3: NSC-58514; CAS reg. No. 7059-24-7
Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6; daunomycin
DDUG: NSC-111056; CAS reg. No. 15427-75-5; carbanilide, 4,4'-diacetyl-, bis(aminohydrazone), dihydrochloride, trihydrate
Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1
Mithramycin: NSC-24559; CAS reg. No. 18378-89-7
Mitomycin C: NSC-26980; CAS reg. No. 50-07-7

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Role of Chemical Structure in Biochemical Activity of Daunorubicin (NSC-82151), Adriamycin (NSC-123127), and Some Structural Analogs: Macromolecular Interactions and Their Biologic Consequences[1]

Prakash Chandra[2,3,4]

Some of the major advances in the chemotherapy of cancer are attributed to the use of chemotherapeutic "cocktails." This expression means that the primary compound is used in combination with other therapeutically active compounds whose mode of action is at least partially known. The idea is to increase the chemotherapeutic efficacy of the primary compound. This can be achieved in one of the following ways: *(a)* by facilitating the favorable distribution of the drug at its site of action; *(b)* by altering the pharmacodynamics of the compound so as to slow down its degradation and allow its existence for a longer period in its therapeutically active form; and *(c)* (perhaps most important, in my opinion) by promoting the multi-site action of the individual components of the cocktail. For such an ideal combination, the mechanism of action of the individual components of the cocktail must be known. This approach should help to reduce the drug toxicity, since in a multi-site action spectrum, therapeutically favorable results might be achieved at reduced dose levels.

Another approach which may lead directly to the potentiation of the chemotherapeutic efficacy of a compound is its structural modification. This has been observed by us with respect to the biochemical and cytostatic activity of daunorubicin, adriamycin, and their structural analogs, which were synthesized in the research laboratories of Farmitalia, Milan, Italy. Our initial studies (1-4) were carried out with daunorubicin derivatives that had substitutions in the anthracycline ring (adriamycin and 13-dihydrodaunomycin) or in the amino sugar moiety (*N*-guanidine-acetamide-daunomycin and *N*-acetyl-daunomycin) (fig 1).[5]

The inhibitory effect of different daunorubicin derivatives (fig 1) on viral oncogenesis by Friend leukemia virus (FLV) and Rous sarcoma virus (RSV) is shown in table 1. FLV suspensions were prepared by filtering the homogenates from the infected spleens of AKR mice through Seitz EK filters (Seitz Co., Bad Kreuznach, Germany).

This suspension was diluted 1:20 with Hank's solution. The diluted suspension was incubated with daunorubicin or its derivatives (50 μg/ml) for 1 hour at 37°C; control suspensions were incubated under similar conditions but without the antibiotic. A total of 0.1 ml of each suspension (ID90) was injected intraperitoneally (ip). Each experimental group contained six animals. As can be seen in table 1, five of six control animals died 13 days after infection. At this time the animals injected with daunorubicin-treated viral suspensions were all alive. Adriamycin also had a very beneficial effect, whereas 13-dihydro-daunomycin showed only moderate activity. The derivatives substituted in the amino sugar moiety were ineffective. Oncogenesis in chickens by RSV was also inhibited by daunorubicin and its analogs, and the mean survival time was prolonged by adriamycin from 12.3 to 28.3 days. Daunorubicin was even more effective, whereas the other derivatives had no significant effect.

The inhibitory activity of daunorubicin and its structural analogs on viral oncogenesis suggests that the virus-associated enzymatic activities are sensitive to these antibiotics. The RNA-dependent DNA polymerase of virions is responsible for the synthesis of DNA chains on the RNA template, giving rise to a hybrid molecule (RNA-DNA). These DNA chains are released from the RNA template as single-stranded DNA molecules and serve as templates for the synthesis of double-stranded DNA. These synthetic processes could be affected by the daunorubicin derivatives. The inhibition of the reverse transcriptase activity of Moloney sarcoma virus (MSV), FLV, and RSV by various daunorubicin derivatives is shown in table 2. Daunorubicin and adriamycin at 10 μg/reaction mixture (0.25 ml) inhibited the DNA-polymerase reaction (endogenous) to 60%-70%. The dihydro derivative was also quite effective whereas the *N*-guanidine derivative had only moderate activity. The *N*-acetyl derivative was completely ineffective in the MSV and FLV systems. The RSV system was moderately inhibited by the *N*-acetyl derivative.

Synthetic polymers containing either deoxyribonucleotide or ribonucleotide strands are known to stimulate in vitro DNA synthesis by RNA tumor

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[1]Daunorubicin: CAS reg. No. 23541-50-6.
Adriamycin: CAS reg. No. 23214-92-8.

[2]Gustav-Embden-Zentrum der Biologischen Chemie, Abteilung für Molekularbiologie, Universität Frankfurt/Main, Germany.

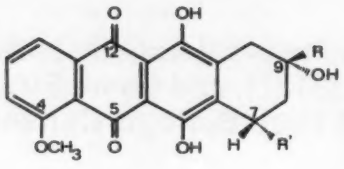
[3]I am much indebted to Drs. F. Zunino and D. Gericke for collaboration in some of the experiments reported in this article. I also gratefully acknowledge Mrs. A. Götz for her expert technical assistance and Mrs. Linda K. Steel for preparing the manuscript.

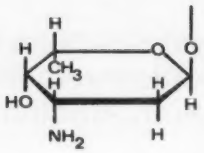
[4]*Reprint requests to:* Dr. Prakash Chandra, Molecular Biology, Theodore-Stern-Kai 7, Frankfurt-Main 70, Germany.

[5]13-Dihydro-daunomycin: NSC-180510; CAS reg. No. 26164-47-6; 5,12-naphthacenedione, 10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(1-hydroxyethyl)-1-methoxy-, (8*S-cis*)-, hydrochloride; duborimycine.

N-acetyl-daunomycin: NSC-118714; α-L-*lyxo*-hexopyranoside, 3β-acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1α-naphthacenyl 3-acetamido-2,3,6-trideoxy-, α-, compd. with isopropyl alcohol (1:1).

General structure





Structure of daunosamine

SUBSTITUTIONS

	R	R
Daunorubicin	$-CO-CH_3$	Daunosamine
Adriamycin	$-CO-CH_2OH$	Daunosamine
13-Dihydro-daunomycin	$-CHOH-CH_3$	Daunosamine
N-guanidine-acetamide-daunomycin	$-CO-CH_3$	N-guanidine-acetamide-daunosamine
N-acetyl-daunomycin	$-CO-CH_3$	N-acetyl-daunosamine

FIGURE 1.—Chemical structures of daunorubicin derivatives.

viruses. Some inhibitors of the DNA-polymerase reaction in RNA tumor viruses are known to exhibit a template-primer specificity (5-7). Table 3 shows the inhibition of poly(dA-dT)-dependent DNA-polymerase activity of FLV by various daunorubicin derivatives.

The reactions catalyzed by poly(dA-dT) are highly sensitive to the action of daunorubicin and its derivatives. Even under these conditions, where daunorubicin and adriamycin are most effective, the *N*-acetyl derivative is completely inactive. It is interesting to note that the poly(dA-dT)-dependent reaction is much more sensitive to antibiotics than the endogenous reaction (table 2).

The results show that daunorubicin requires specific structural parameters for its inhibiting activity. Thus, substitutions in the amino sugar moiety, especially *N*-acetylation, inhibit its activity against oncogenic viruses and influence its inhibitory action on the DNA polymerase of RNA tumor viruses.

The data reported above show that the poly(dA-dT)-catalyzed reaction of viral DNA polymerase is most sensitive to daunorubicin and adriamycin. It is therefore possible that the biologic activity of these

TABLE 1.—Effect of daunorubicin and its derivatives on viral oncogenesis in mice and chickens

Antibiotic	Oncogenesis in mice by FLV (No. of animals surviving*/ No. of animals infected)	Oncogenesis in chickens by RSV† (mean survival time in days)
None	1/6	12.3
Daunorubicin	6/6	30
Adriamycin	5/6	28.3
13-Dihydro-daunomycin	3/6	14.2
N-guanidine-acetamide-daunomycin	1/6	13.6
N-acetyl-daunomycin	0/6	12.0

*13 days after infection; for details see text.

†Each experimental group contained 6 chickens. The tumor suspension (1:10) was incubated with 50 μg/ml of antibiotic at 37°C for 1 hr. Control suspension was incubated without the antibiotic. 0.1 ml of this suspension was injected ip.

TABLE 2.—Inhibition of DNA-polymerase activity* (endogenous) of RNA tumor viruses by daunorubicin derivatives

System	Antibiotic†	^{3}H-TMP incorporation into DNA‡ (cpm/reaction mixture) MSV	FLV	RSV
Without virions	—	7 (3.4)	7 (3.7)	7 (2.9)
Virions + RNase§	—	26 (13)	25 (13.4)	40 (16.8)
Complete	None	202 (100)	187 (100)	237 (100)
	Daunorubicin	65 (32.1)	68 (36.3)	80 (33.7)
	Adriamycin	66 (33.1)	83 (44.4)	86 (36.3)
	13-Dihydro-daunomycin	86 (42.5)	87 (46.5)	113 (47.7)
	N-guanidine-acetamide-daunomycin	106 (52.4)	97 (51.8)	117 (49.3)
	N-acetyl-daunomycin	196 (97)	192 (102.6)	136 (57.3)

*DNA-polymerase assay: DNA-polymerase activity was assayed essentially by the method of Ross et al (9). The reaction mixture, regardless of template, was similar to that of Ross et al except that we used 0.04 M tris and the end concentration of Nonidet P-40 (Shell Chemie, Hamburg) was 0.2%. The reaction mixture contained 0.25 μg of the template used. The reaction mixture (0.25 ml) containing virions (28 μg of protein) was incubated at 37°C for 90 mins. Acid precipitable material was counced on Millipore filters (HAWP 02500) in a liquid scintillation counter. Protein was estimated by the method of Lowry et al (8).

†Antibiotic concentration = 10 μg/reaction mixture (0.25 ml).

‡Figures in parentheses = % of control.

§Virions containing Nonidet P-40 were preincubated at room temperature for 25 mins with 50 μg/ml of pancreatic RNase.

compounds is due to their interaction with the hybrid RNA-DNA (hy-DNA), the single-stranded DNA (ss-DNA), or the double-stranded DNA duplex (ds-DNA). Experiments were conducted to locate the site of action of adriamycin in the viral DNA-polymerase system from FLV.

We have conducted some model studies to analyze the products of FLV-DNA-polymerase reaction under the influence of adriamycin. The procedure we adopted was based on a recent report by Kotler and Becker (10) on distamycin A, which has been shown by us to react with ss-DNA and ds-DNA (11).

The product analysis of the DNA-polymerase reaction (FLV) in the absence and in the presence of adriamycin (20 μg/ml) is depicted in figure 2. Under these conditions, the products of the viral DNA-polymerase reaction were eluted in three species. The first species to be eluted from the column contained ss-DNA, the second contained hy-DNA, and the third contained ds-DNA. Analysis of the products synthesized in the presence of adriamycin showed that the ss-DNA and the hy-DNA, but not the ds-DNA species, were synthesized. This indicates that adriamycin has no affinity for viral RNA but can block the synthesis of ds-DNA by interacting with ss-DNA or hy-DNA.

To evaluate the therapeutic efficacy of these compounds, the effect of daunorubicin and adriamycin on DNA polymerases from various sources was measured. These studies were carried out by using a constant concentration of the template poly(dA-dT) in DNA-polymerase reactions catalyzed by preparations from MSV, rat liver, and *Micrococcus lysodeikticus*. The results in figure 3 show that the MSV DNA polymerase is the most sensitive to both antibiotics.

The antitumor activity of some derivatives of daunorubicin at the amino and methyl-ketone functions has been studied by Yamamoto et al (12). Their studies were carried out mainly on mice with L1210 leukemia. At a dose of 2 mg/kg, the *N*-piperidinoimine derivative was found to have the same

TABLE 3.—Inhibition of DNA-polymerase activity of FLV by daunorubicin and its derivatives in the presence of poly(dA-dT)

Antibiotic*	^{3}H-TMP incorporation into DNA (cpm/reaction mixture)	% of control
None	1223	100
Daunorubicin	127	10.3
Adriamycin	106	8.7
13-Dihydro-daunomycin	151	12.3
N-guanidine-acetamide-daunomycin	322	26.3
N-acetyl-daunomycin	1412	115.6

*Antibiotic concentration = 5 μg/reaction mixture (0.25 ml). Reaction conditions are shown in table 2.

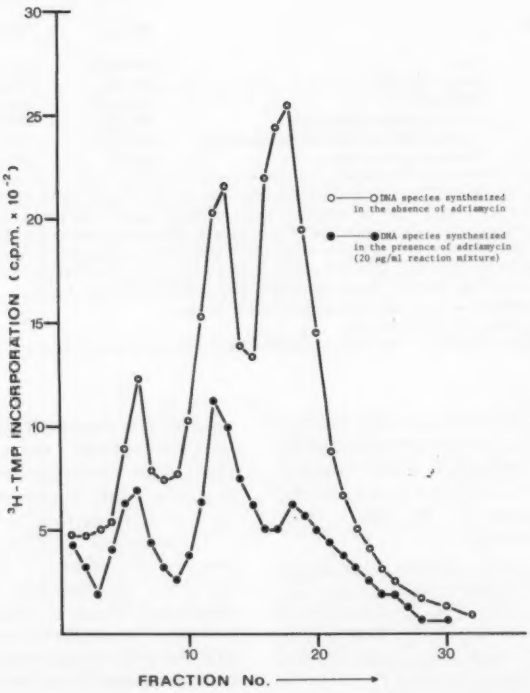


FIGURE 2.—Analysis of the DNA species synthesized by FLV-DNA polymerase by elution from hydroxylapatite column. Each column was filled with 1 g of hydroxylapatite and carefully washed with 0.05 M sodium phosphate buffer (pH 6.8) (approximately 50 ml). The columns were loaded with the reaction products dissolved in dodecyl sulfate (10). The columns were washed with 0.05 M sodium phosphate buffer (pH 6.8) until equilibrium was reached. Macromolecules were eluted from the column by a linear gradient of sodium phosphate buffer (0.05-0.4 M).

antitumor activity as daunorubicin; other derivatives were not active at this dose level. The *N*-acetyl derivative was found to possess a little antitumor activity, but displayed no acute toxicity even at very high doses. According to our experience, the *N*-acetyl derivative was in most of the cases ineffective against tumor growth (table 4).

As is clear from table 4, adriamycin inhibits the growth of Ridgway osteosarcoma in mice by more than 80%. Under similar experimental conditions one finds a slight inhibitory effect by daunorubicin and its 13-dihydro derivative. However, the derivatives with substitutions in the amino sugar moiety, *N*-guanidine-acetamide-daunomycin and *N*-acetyl-daunomycin, are completely ineffective. In the L1210 and SV40 systems, tumor suspensions preincubated with adriamycin or daunorubicin failed to grow in their hosts. The 13-dihydro derivative was not effective in L1210 but total inhibition was achieved in SV40. Unexpectedly, the *N*-guanidine-acetamide derivative showed significant activity against the SV40 tumor system. The *N*-acetyl derivative was ineffective against all types of tumors studied by us.

The structure-activity relationship exhibited in enzymatic and biologic systems was also observed with respect to their physicochemical interaction with DNA (table 5). Daunorubicin causes a large increase in the thermal transition temperature (T_m) of calf thymus DNA. This effect depends on the ratio of antibiotic to DNA-P(r). The effect of daunorubicin derivatives on the T_m of calf thymus DNA at $r = 0.1$ is shown in table 5. Adriamycin was found to be most effective in stabilizing the secondary structure of

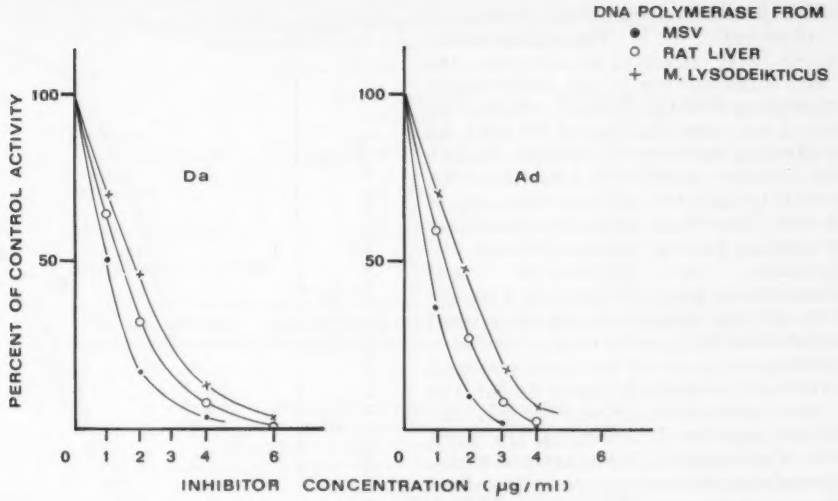


FIGURE 3.—Inhibitory effect of daunorubicin (Da) and adriamycin (Ad) on DNA polymerases from three different sources. For details *see* text (*from* ref 5).

TABLE 4.—Inhibition of growth of some transplanted tumors by daunorubicin derivatives*

Antibiotic	Ten animals transplanted with— Ridgway osteosarcoma (mouse) (tumor weight, g)	L1210 leukemia (mouse) (ascites, ml)	SV40 (hamster) (tumor weight, g)
None	7.0 ± 2.4	0.78 ± 0.16	47 ± 12
Adriamycin	1.0 ± 0.3	0.00	0.00
Daunorubicin	5.0 ± 2.3	0.00	0.00
13-Dihydro-daunomycin	5.3 ± 2.0	0.53 ± 0.2	0.00
N-guanidine-acetamide-daunomycin	7.8 ± 2.8	0.63 ± 0.1	18.0 ± 8
N-acetyl-daunomycin	9.8 ± 1.4	0.64 ± 0.07	46 ± 8

*Tumor suspensions were incubated with 50 μg/ml of the antibiotic at 37°C for 1 hr.

TABLE 5.—Effect of daunorubicin and its derivatives on the thermal transition temperature (T_m) and viscosity of DNA*

Antibiotic	T_m	$\frac{(\eta)r = 0.1}{(\eta)r = 0}$ †
None	70.5	1.00
Adriamycin	85.8	1.75
Daunorubicin	83.9	1.92
13-Dihydro-daunomycin	80.3	1.65
N-guanidine-acetamide-daunomycin	77.1	1.30
N-acetyl-daunomycin	71.5	1.24

*All experiments were carried out in 0.01 M tris-HCl buffer (pH 7.0) at a ratio of antibiotic to DNA-P(r) of 0.1. DNA concentration in all experiments was 1×10^{-4} M.

†Ratio of intrinsic viscosity of antibiotic-DNA complex (r = 0.1) to that of DNA alone. Conditions of viscosity measurements: 20°C, 0.1 M tris-HCl buffer (pH 7.0). r = ratio of bound antibiotic to total DNA-P.

DNA (T_m = 15.3°C), whereas very little increase in T_m was observed for *N*-guanidine-acetamide-daunomycin and *N*-acetyl-daunomycin, the derivatives with substitutions in the amino sugar moiety. In attempting to obtain further information on the affinity of the compounds tested for DNA, we studied their effect on the viscosity of DNA. Table 5 also shows the intrinsic viscosity of antibiotic-DNA complexes (r = 0.1) relative to intrinsic viscosity of DNA alone (r = 0). Under these conditions the daunorubicin-DNA complex has the highest intrinsic viscosity, followed by adriamycin and 13-dihydro-daunomycin. Again we find only a moderate increase in intrinsic viscosity in the presence of compounds substituted at the amino sugar residue.

Because adriamycin is one of the most powerful among the cytostatic compounds tested so far, the synthesis of some adriamycin derivatives was carried out in the laboratories of Farmitalia. The chemical structures of adriamycin-14-acetate and adriamycin-14-octanoate are shown in figure 4.[6] The effect of adriamycin and adriamycin-14-acetate on the T_m of calf-thymus DNA at a drug:DNA-P(r) ratio of 0.1 is shown in figure 5, and that of adriamycin and adriamycin-14-octanoate is shown in figure 6. The rise in T_m of calf thymus DNA in the presence of adriamycin (curve 2) and adriamycin-14-acetate (curve 3, fig 5) exhibits a similar profile whereas under similar experimental conditions there was a large difference between adriamycin (curve 2) and adriamycin-14-octanoate (curve 3, fig 6).

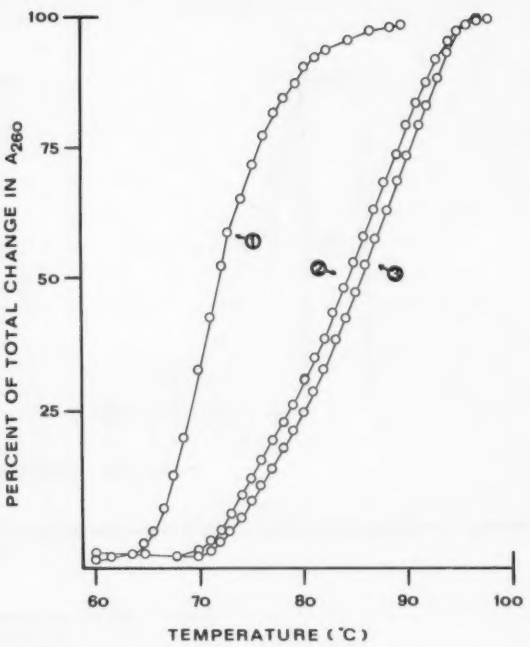
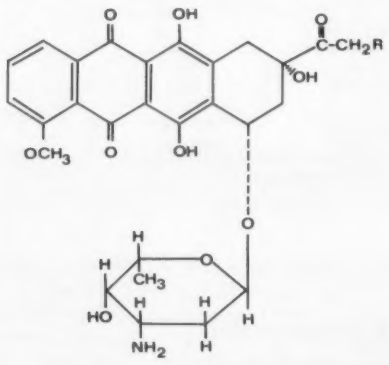


FIGURE 5.—Thermal transition curves of DNA alone (curve 1), DNA-adriamycin complex at r = 0.1 (curve 3), and DNA-adriamycin-14-acetate at r = 0.1 (curve 2).

[6]Adriamycin-14-acetate: NSC-149581; CAS reg. No. 41962-27-0; adriamycin, 14-acetate, hydrochloride.

Adriamycin-14-octanoate: NSC-149584; adriamycin, 14-octanoate, hydrochloride.



Daunorubicin	R = H
Adriamycin	R = OH
Adriamycin 14-Acetate	R = $OCOCH_3$
Adriamycin 14-Octanoate	R = $OCO(CH_2)_6CH_3$

FIGURE 4.—Chemical structures of some adriamycin derivatives.

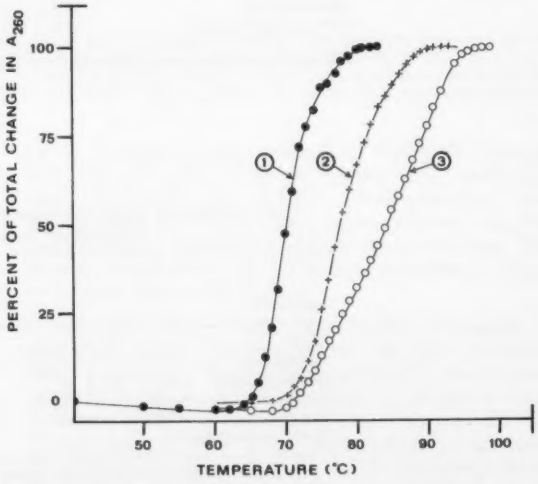


FIGURE 6.—Thermal transition curves of DNA alone (curve 1), DNA-adriamycin complex at r = 0.1 (curve 3), and DNA-adriamycin-14-octanoate at r = 0.1 (curve 2).

TABLE 6.—Inhibition of FLV-DNA-polymerase reaction by adriamycin derivatives [template = poly(dA-dT) • poly(dA-dT)]

Antibiotic	Concentration of compound (1×10^{-5} M)	^{3}H-TMP incorporation into DNA (% of control)
Control	—	100 (3789)*
Adriamycin	0.5	29
	1.0	6.2
Adriamycin-14-acetate	0.5	39.0
	1.0	8.0
Adriamycin-14-octanoate	0.5	94.0
	4.0	47.8

*No. in parentheses = cpm/reaction mixture (0.25 ml) incorporated in 60 mins. The reaction mixture contained 1 μg of the template.

TABLE 7.—Effect in vitro of adriamycin derivatives on the leukemogenic potential of cell-free spleen extracts prepared from spleens of FLV-infected mice (AKR strain)

Antibiotic	Spleen weight (g) for 4 animals per group 12 days after infection
Control (infected)	3.75, 2.56, 1.78, 3.51 (2.89)†
Adriamycin (5×10^{-4} M)	2.21, 1.37, 0.93, 0.33 (1.19)
Adriamycin-14-acetate (5×10^{-4} M)	0.92, 1.10, 0.43, 1.98 (1.10)
Adriamycin-14-octanoate (5×10^{-4} M)	2.29, 1.88, 2.28, 0.45 (1.72)

*Preincubation of cell-free extracts, with or without compound, was carried out for 2 hrs at 37°C.

†Figures in parentheses are the arithmetic means of individual values.

The same structure-activity relationship could be seen in the poly(dA-dT)-catalyzed DNA-polymerase reaction in FLV (table 6). Adriamycin inhibits this reaction by about 70% at 5×10^{-6} M, whereas its octanoate derivative was almost ineffective; under similar conditions, adriamycin-14-acetate showed about 60% inhibition.

It should be noted that the data on the in vitro activity of adriamycin derivatives presented above are not consistent with their cytostatic activity against FLV. The structure-activity relationship of adriamycin derivatives in the enzymatic system and with respect to their interaction with DNA is not observed in the biologic system. The in vitro effect of adriamycin derivatives on the leukemogenic potential of cell-free spleen extracts prepared from spleens of FLV-treated mice is shown in table 7; it is clear that the octanoate derivative is very effective in inhibiting FLV leukemogenesis. Goldin and Johnson (13) compared the activity of adriamycin and adriamycin-14-octanoate against Lewis lung carcinoma and found that both compounds have a similar effect.

The differences between the biologic activity of adriamycin derivatives and their in vitro effects on DNA structure indicate that these derivatives undergo some metabolic alteration leading to the formation of a derivative that behaves in a similar way to adriamycin. As recently observed by Lenaz,[7] the octanoate derivative is converted in vivo to hydroxy-adriamycin. This explains the significant activity of the octanoate derivative in biologic systems, despite its very moderate interaction with DNA under in vitro conditions.

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[7]Lenaz L (Farmitalia, Milan, Italy). Personal communication.

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Adriamycin (NSC-123127): New Chemical Developments and Analogs[1]

Federico Arcamone, Sergio Penco, and Aristide Vigevani[2,3]

The discovery (1) of adriamycin (I, fig 1), the conclusion of the chemical studies leading to the elucidation of the structure and stereochemistry of the antitumor anthracyclines (2-5), and the establishment of the effectiveness of adriamycin in the treatment of human malignancies (6) have opened a new field in medicinal chemistry. During recent years, different important contributions to the chemistry of the anthracyclines have appeared in the scientific literature. Among these, particular mention should be made of the total synthesis of racemic daunomycinone (III) and racemic isodaunomycinone (IV) described by Wong et al in 1973 (7) (fig 2). The same synthetic approach, in which the tetracyclic ring system is synthesized following the known scheme (8,9) based on the acylation of a substituted tetraline with a phthalic acid derivative and ring closure under strong acidic conditions, had also been previously used for the synthesis of the two related aglycones, V (10) and VI (11) (fig 3). A Diels-Alder reaction has been used for the synthesis of the anthracyclinone ring system by Fariña and Vega (12) and by Lee et al (13). A regiospecific synthesis of an unsymmetrically substituted anthraquinone ultimately leading to the olefinic precursor of daunomycinone (VII) has been reported by Kende et al (14) (fig 4).

Another point which has received consideration is the synthesis of the glycosidic bond between the aglycone and the sugar. That the Koenigs-Knorr reaction was a useful method for the coupling of a suitably protected glycosyl halide to daunomycinone or related aglycones had been shown by Penco (15) in the synthesis of the glycosides VIII and IX and by Marsh et al (10) in the synthesis of the two diastereoisomeric compounds of the general structure X (fig 5). Recently, Acton et al (16) have reported the Koenigs-Knorr condensation of daunomycinone with 4-*O*-*p*-nitrobenzoyl-3-*N*-trifluoroacetyldaunosaminyl bromide to produce daunorubicin[4] (II) after removal of the protecting groups. Because the total synthesis of daunomycinone (7), the preparation of daunosamine from L-rhamnose (17), and the conversion of daunorubicin to adriamycin (18) had already been described, a formal synthesis of adriamycin was thus completed. The same coupling reaction has been used by Fujiwara et al (19) for the synthesis of the analog XI and its C-7 epimer. Compound XI (fig 6) represents the first example of a new semisynthetic anthracycline analog in which the natural amino sugar, daunosamine, is linked to a modified aglycone obtained by total synthesis.

These synthetic studies are important for different reasons. Total synthesis of the antitumor anthracyclines could furnish an alternative method, possibly competitive with the biosynthetic process, for the preparation of adriamycin. However, it should also lead to a variety of new structurally related analogs to be tested for antitumor activity. Although a total synthesis of adriamycin has already been formally achieved, the former possibility seems remote at the present time. The second possibility (ie, the development of new analogs) is already partially achieved, and current work in this area will certainly give the desired results within a short time.

Chemical modification of daunorubicin and adriamycin represents an obvious approach to the development of new antitumor agents. Derivatives at the side chain carbonyl or at the amine function of daunorubicin, as well as esters at the C-7 position of daunomycinone, have been prepared (20-22). Potassium borohydride reduction of the side-chain carbonyl of both daunorubicin and adriamycin has been reported (23). 14-Bromodaunomycin (XII, R = Br) has been found to be a useful intermediate for the derivatization of the side chain with suitable nucleophiles (fig 7). Substitution of the halogen by a hydroxyl produced adriamycin (24), whereas reaction with the sodium or potassium salts of organic acids yielded the 14-*O*-acyl derivatives of adriamycin (XII, R = acyloxy) (24, 25). Recently, Israel et al (26) described a number of alkanoic esters of the hydroxymethylketone function of *N*-trifluoroacetyladriamycin (XIII, R = alkyl) (fig 7).

The structure and stereochemistry of the amino sugar moiety of daunorubicin and adriamycin are important requirements for the formation of a stable complex between the antibiotics and DNA and, therefore, for the exhibition of those biochemical interactions which are a consequence of the complex formation (27). It may also be assumed that the structure and stereochemistry of the carbohydrate portion of the molecule are of primary importance in determining the pharmacokinetic behavior and metabolism of the biologically active glycosides. It is clear that all of the above-mentioned properties directly influence the pharmacologic activity. We now wish to report the synthesis of new glycosides which are stereoisomers of daunorubicin or of adriamycin. With respect to the natural antibiotics, the new glycosides have a different absolute configuration at position 1 and/or 4 of the sugar moiety. This work is part of a program directed towards the semisynthesis of analogs of the antitumor anthracyclines modified in the daunosamine residue.

[1]Adriamycin: CAS reg. No. 23214-92-8.

[2]Farmitalia, Milan, Italy.

[3]*Reprint requests to:* Dr. Federico Arcamone, Farmitalia, Viale E Bezzi 24, 20146 Milan, Italy.

[4]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

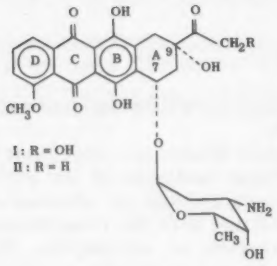
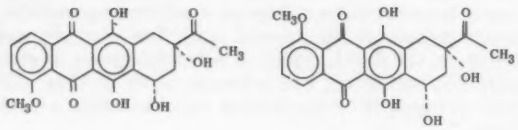
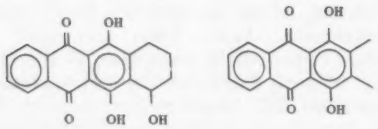


FIGURE 1.



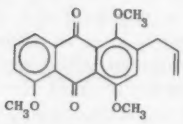
III IV

FIGURE 2.



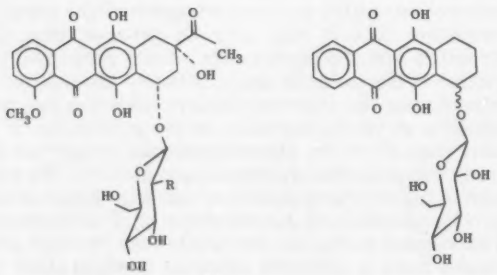
V VI

FIGURE 3.



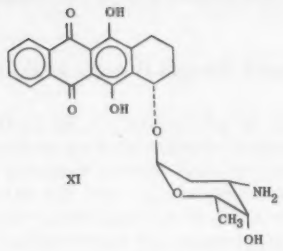
VII

FIGURE 4.



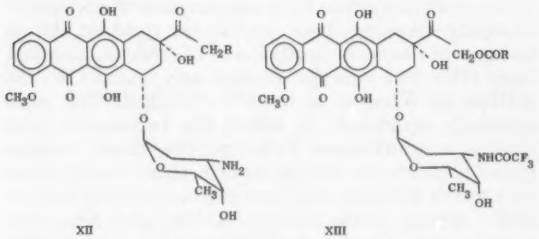
X

FIGURE 5.



XI

FIGURE 6.



XII XIII

FIGURE 7

The steric course of the Koenigs-Knorr reaction is known to be dependent on different factors operating on a complex mechanism which is presently not completely understood (28). In the absence of neighboring participating groups, the relative amounts of α- and β-anomers are dependent, inter alia, on the reaction conditions and on the halo sugar used.

We have used *N,O*-ditrifluoroacetyl-α-daunosaminyl chloride (XIV) as the glycosylating agent in the Koenigs-Knorr synthesis of the glycosidic bond of daunorubicin and adriamycin. The instability of the 1-halo-2-deoxy sugars is well documented (29), but *N,O*-ditrifluoroacetyl-α-daunosaminyl chloride appears to be endowed with sufficient stability to be stored under anhydrous conditions for several days. The proton magnetic resonance (pmr) spectrum ($CDCl_3$) of the product is shown in figure 8. The most important signals are the broad singlet of the equatorial anomeric proton at 6.29 δ (half-band width about 6 Hz), the broad singlet of H-4_{eq} at 5.37 δ (half-band width about 6 Hz), and the quartet of H-5_{ax} at 4.46δ. The four lines of the quartet are broadened, due to the small vicinal coupling (<1 Hz) between H-5_{ax} and H-4_{eq}.

When *N,O*-ditrifluoroacetyl-α-daunosaminyl chloride (1.3 mM) is added to a stirred mixture of daunomycinone (1 mM), mercuric oxide (3.5 mM), and mercuric bromide (1.1 mM), and molecular sieve in dichloromethane at room temperature, and the reaction is followed by thin-layer chromatography, the spot corresponding to the aglycone is reduced to approximately 10% within 16 hours and two less polar products are formed. Recovery of the reaction

products followed by hydrolysis of the *O*-trifluoroacetate grouping after treatment with methanol produced two products appearing now as more polar species with respect to daunomycinone, corresponding respectively to the α- and β-*N*-trifluoroacetyl glycosides in the approximate ratio of 7:3. Chromatographic separation gave *N*-trifluoroacetyldaunorubicin (18) and the corresponding β-anomer (XV) [mp = 138°-140°, $[\alpha]_D^{20}$ = +440° (c 0.1 $CHCl_3$)] (fig 9). Mild alkaline treatment of *N*-trifluoroacetyldaunorubicin produced daunorubicin (II), while XV yielded 7-*O*-β-daunosaminyldaunomycinone (XVI, fig 9).

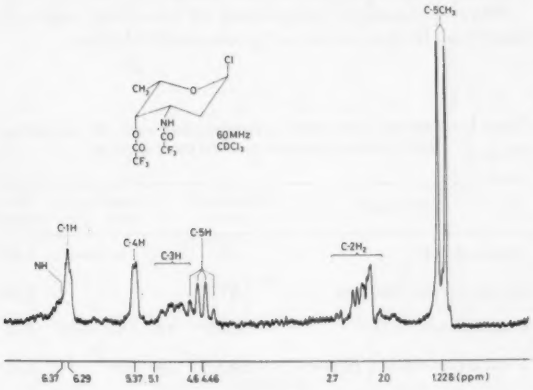
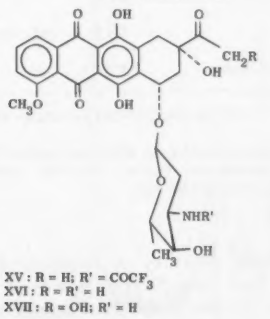


FIGURE 8.—Pmr spectrum of *N,O*-ditrifluoroacetyldaunosaminyl chloride (XIV).



XV : R = H; R' = $COCF_3$
XVI : R = R' = H
XVII : R = OH; R' = H

FIGURE 9

In order to perform the coupling of daunosamine with adriamycinone, the hydroxymethylketone function of the latter was protected as indicated in XVIII (fig 10).

Compound XVIII was obtained by treatment of adriamycinone with 2,2-dimethoxypropane under anhydrous acid conditions, and its mass spectrum is presented in figure 10. The more significant peaks correspond to the molecular peak (m/e 486), and to the ions derived from the loss of methanol (m/e 454) and of two molecules of water (m/e 450). The abundant fragment at m/e 131 corresponds to the substituted dioxolanyl ion.

Treatment of XVIII with XIV under the above-mentioned conditions and then with methanol yielded the two anomeric glycosides XIX and XX (fig 11). Removal of the protecting groups using mild alkaline treatment and then acid produced adriamycin (from XIX) and the corresponding β-anomer XVII (from XX) (fig 9).

The α- and β-anomers in the daunorubicin-adriamycin series are easily identified, inter alia, by pmr spectroscopy. For example, the axial orientation of the anomeric proton in XX is shown by the fact that the corresponding signal (60 MHz, $CDCl_3$) is a pair of doublets with coupling constants of 2 and 10 Hz, whereas the equatorial anomeric proton in XIX appears as a broad singlet with a half-band width of about 6 Hz. The H-1′ signal in XX shifts to higher field values (about 0.5 δ) with respect to the α-anomer XIX, which displays the H-1′ signal at

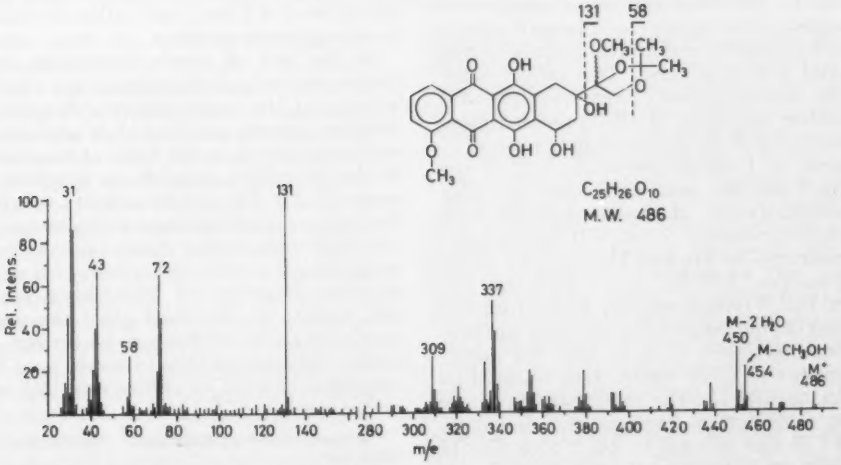


FIGURE 10.—Mass spectrum of 9-desacetyl-9-(2,2′-dimethyl-4-methoxydioxolane-4′-yl)-daunomycinone (XVIII).

about 5.5 δ. These data are in agreement with the observations made on the α- and β-anomeric methyl-*N*-trifluoroacetyldaunosaminides; in these compounds the anomeric proton appears, for the α-anomer, as a broad singlet at lower field (4.75δ) than the pair of doublets of the β-anomer (4.45δ, J = 2.5 and 9.5 Hz). Instead, the reverse phenomenon is observed for the benzylic proton at C-7, the H-7 signal appearing in the range of 5.0δ-5.2δ in the α-series and about 5.5δ in the β-series. Also in this case the behavior is similar to that displayed by the methoxy groups of anomeric methyl-*N*-trifluoroacetyldaunosaminides, in which the signal of the axial methoxy group (α-anomer) appears at higher field (3.38δ) than that of the equatorial group (β-anomer, 3.53δ) (30) The anomeric axial methoxyl group also resonates at higher field in the glucose series (31,32).

As with the daunorubicin and adriamycin syntheses reported above, we have used 3-amino-*N*,*O*-ditrifluoroacetyl-2, 3, 6-trideoxy-α-L-arabino-hexopyranosyl chloride (XXVII, fig 12) as the glycosylating agent for the synthesis of the corresponding glycosides with L-arabino configuration. The preparation of the halo sugar intermediate was performed starting from α-methyldaunosaminide (XXI) which was converted to the *N*-trifluoroacetyl derivative (XXII) and oxidized with ruthenium tetroxide to produce methyl-2,3,6-trideoxy-3-trifluoroacetamido- α - L - threo-hexopyranoside-4-ulose (XXIII, fig 12). Reduction of XXIII with sodium borohydride at +5°C resulted in the stereoselective formation of the equatorial alcohol XXIV (fig 12). The 4-*O*-mesyl derivative of the *N*-acetyl analog of XXIV had been obtained previously by Marsh et al (17) as an intermediate in the synthesis of daunosamine. *N*-trifluoroacetyl-4-*epi*-daunosamine (XXV), obtained upon hydrolysis of XXIV, was trifluoroacetylated to XXVI and then converted to XXVII with dry hydrogen chloride (fig 12).

The pmr spectrum of XXIV (DMSO-d_6 + D_2O) is shown in figure 13. The most significant signals are the broad singlet of the equatorial anomeric proton at 4.48δ (half-band width about6 Hz) and the triplet of the C-4 axial proton at 3.0 δ. This triplet originates from the superposition of two doublets with identical coupling constants of 10 Hz and indicates the axial orientation of the corresponding proton.

The synthesis of 4′-*epi*-daunorubicin and of the corresponding β-anomer was performed by Koenigs-Knorr condensation of daunomycinone with XXVII followed by removal of the *O*-trifluoroacetyl group with methanol to produce the mixture of the anomeric glycosides XXVIII and XXXI (fig 14). Hydrolysis of the *N*-trifluoroacetyl group followed by a chromatographic separation on silica gel produced 4′-*epi*-daunorubicin (XXIX) and the corresponding β-anomer (XXXII) which were isolated as the hydrochlorides (fig 14). For the synthesis of the corresponding adriamycin analogs the dioxolane derivative (XVII) was allowed to react with XXVII under the conditions of the Koenigs-Knorr condensation. The resulting products were methanolyzed to produce the mixture of XXXIV and XXXV which were isolated by silica gel chromatography and treated separately with dilute sodium hydroxide and then with acid to give 4′-*epi*-adriamycin (XXX) and the β-anomer (XXXIII) respectively (fig 14).

Physicochemical properties of the new analogs described in this paper are presented in table 1.

TABLE 1.—Melting points (mp) and chromatographic R_f values for daunorubicin, adriamycin, and their analogs

Compound	Structure No.	mp* (C)	R_f value†
Daunorubicin	II	188°-190° (dec)	0.63
Daunorubicin, β-anomer	XVI		0.50
4′-*epi*-Daunorubicin	XXIX	199°-201° (dec)	0.65
4′-*epi*-Daunorubicin, β-anomer	XXXII	187°-189°	0.60
Adriamycin	I	205° (dec)	0.45
Adriamycin, β-anomer	XVII		0.35
4′-*epi*-Adriamycin	XXX	185° (dec)	0.55
4′-*epi*-Adriamycin, β-anomer	XXXIII		0.43

*Determined by the Kofler microscopic hot-stage method and uncorrected.

†Thin-layer chromatography on silica gel plates buffered at pH 7 with M/15 phosphate buffer. Solvent system: chloroform:methanol:water (13:6:1 by vol).

In vivo biologic activity of compound X is still unreported. It has been found, however, that both the (S) and the (R) diastereoisomers are equally active as inhibitors of DNA synthesis in L1210 cells, although distinctly less active than adriamycin (33). The (S) diastereoisomer differs from adriamycin in the absence of functionalization at positions 4 and 9 on the aglycone moiety.

In the field of simple functional derivatives of daunorubicin and adriamycin, the retained biologic activity of the methylketone side-chain derivatives and the reduced activity of *N*-acylated derivatives can be explained on the basis of the molecular model of the drug-DNA complex, as observed in other reports (27,33). The in vivo activity reported for some derivatives, such as daunomycin-benzoylhydrazone[5] (in which encouraging clinical results in acute leukemias [34] but not in solid tumors [35] were described) and the 14-esters of *N*-trifluoroacetyladriamycin (26), should be discussed after the necessary adequate metabolic studies are performed. Biologically active adriamycin-14-octanoate[6] was found to be converted in vivo to adriamycin (25) and this phe-

[5]Daunomycin-benzoylhydrazone: NSC-164011; CAS reg No. 36508-71-1.

[6]Adriamycin-14-octanoate: NSC-149584; adriamycin, 14-octanoate, hydrochloride.

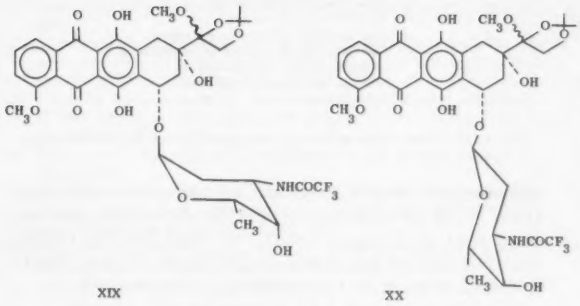


FIGURE 11

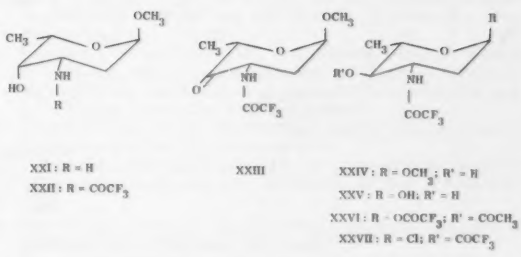


FIGURE 12

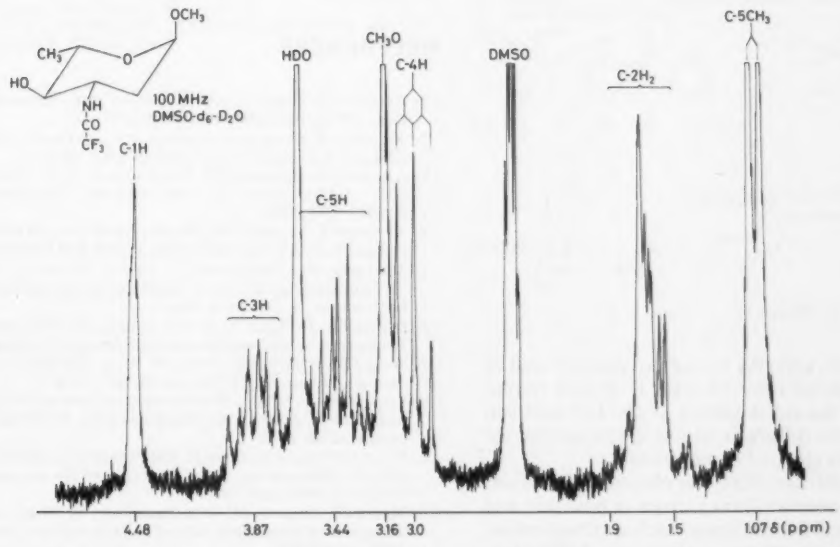


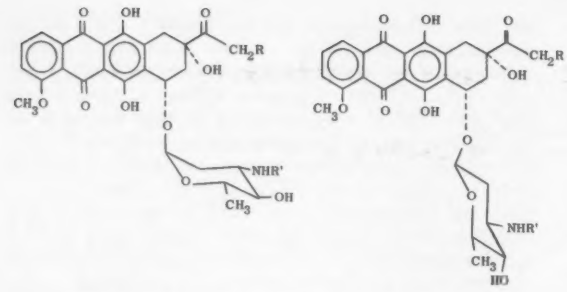
FIGURE 13.—Pmr spectrum of methyl-2,3,6-trideoxy-3-trifluoroacetamido-α-L-arabinohexopyranoside.

nomenon, together with the different pharmacokinetic behavior, makes it very difficult to establish structure-activity relationships at the animal level.

Structure-activity relationships are of considerable importance for the establishment of structural requirements for action and, therefore, contribute to the understanding of the fundamental biochemical processes which represent the basis of the mechanism of action of drugs. In the field of the antitumor anthracyclines, we think that very useful information can be obtained by new compounds such as XVI, XVII, XXIX, XXX, XXXII, and XXXIII, in which only minor modifications in the basic adriamycin structure are introduced while all the typical chemical functions of the drug are retained. Biologic activity of the new glycosides is presently being evaluated by Di Marco et al at the Istituto Nazionale Tumori, Milan, Italy. Preliminary results indicate that (*a*) the analogs modified in the stereochemistry at the C-4′ and/or the C-1′ position are still bioactive, (*b*) the compounds presented in table 1 display nonidentical ratios of effective doses in different tests thus suggesting the possibility of different selectivity of action, and (*c*) 4′-*epi*-adriamycin appears to possess the same degree of activity as adriamycin in some experimental tumors in mice but a lower cytotoxic activity in cultured cells.

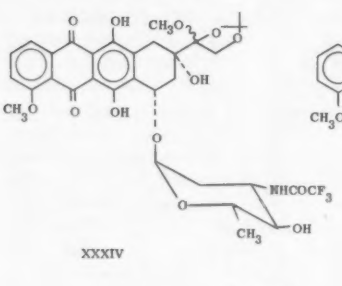
Interestingly, the inversion of configuration at the C-1′ position is not accompanied by a loss of the known DNA complexing ability of the antitumor anthracyclines as indicated by the spectroscopic behavior of the β-anomer of adriamycin in the presence of a commercial preparation of native DNA.

The marked effect of carminomycin (36) on DNA synthesis in L1210 cultured cells (33) is interesting because the methoxyl group on ring D, typical of the daunorubicin-adriamycin subgroup of the anthracyclines, is absent in this compound as well as in the other anthracyclines which display less favorable pharmacologic properties (37). Further investiga-

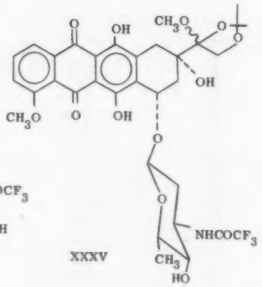


XXVIII : R = H; R' = $COCF_3$
XXIX : R = R' = H
XXX : R = OH; R' = H

XXXI : R = H; R' = $COCF_3$
XXXII : R = R' = H
XXXIII : R = OH; R' = H



XXXIV



XXXV

FIGURE 14

tion of the biologic activity of carminomycin[7] and of new analogs modified only on ring D should reveal the relevance of the substitution at the C-4 position with regard to the different biologic properties exhibited within this class of compounds.

This report indicates that the chemical modification of the antitumor anthracyclines is possible and that it can follow different lines, such as those related to total synthesis of new aglycones, derivatization of the daunorubicin or adriamycin molecule, or semisynthesis of new glycosides. This work, which is obviously directed towards the discovery of new antitumor agents endowed with improved pharmacologic properties, is made difficult by the fact that the mechanisms by which adriamycin displays both its noticeable therapeutic activity and its toxic effects are not completely understood at the present time. This means that currently, the medicinal chemist cannot expect to work on the basis of more or less established structure-activity relationships. We are aware that structural requirements for each of the parameters shown in figure 15 (and probably for others which are not considered) should be established before chemical synthesis of new analogs can be optimalized. On the other hand, the synthesis of new compounds and the development of the techniques for the study of each of the above-mentioned

[7]Carminomycin: NSC-180024; 5,12-naphthacenedione, 8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoyl)oxy]-7, 8, 9, 10-tetrahydro-1,6,8,11-tetrahydroxy-, (8*S*-*cis*).

1. Uptake into tumor tissue
2. Binding to DNA
3. Interaction with DNA function
4. Metabolic transformations
5. Pharmacologic activity of metabolites
6. Pharmacokinetics (absorption, distribution, excretion) of drug and metabolites
7. Effect on immunologic responses
8. Cardiotoxicity

FIGURE 15.—Parameters for evaluating efficiency of new analogs.

parameters should give us the requested information. Work is continuing in this direction, and we hope that in the near future we shall be able to discuss the analog approach on the basis of more clearly established structure-activity relationships.

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DNA-Adriamycin Complex: Preliminary Results in Animals and Man[1,2]

M. Rozencweig, Y. Kenis, G. Atassi, M. Staquet, and M. Duarte-Karim[3,4,5]

SUMMARY

The previously assessed superiority of the DNA-adriamycin complex over adriamycin alone in L1210 murine leukemia depends on the strain of mice, the line of L1210, and the route of tumor cell inoculation. In a phase I trial, 6-12-hour infusions of the DNA-adriamycin complex were given at a dosage schedule of 75 mg/m^2 every 3 weeks. The subacute drug-induced toxic effects are similar to those encountered after direct injection of the free adriamycin. Cardiac toxic effects could not be evaluated accurately in this trial. A preliminary pharmacokinetic study demonstrates, in mice as well as in man, striking differences between free and bound adriamycin with regard to initial plasma levels and pattern of clearance from the plasma. These findings are not yet completely understood.

[Cancer Chemother Rep Part 3, vol 6:131-136, 1975]

According to the lysosomotropic drug concept, drugs firmly bound with an "endocytable" carrier do not penetrate into most cells of the body by a mere diffusion mechanism but are taken up by endocytosis only in cells sufficiently endowed with endocytic activity. If a release of the free original drug is obtained inside the cells after lysosomal action, theoretically it can be expected that the activity and the toxicity of such lysosomotropic drugs will be restricted to the cells which, like some tumor cells, simultaneously possess high mitotic rates and endocytic activity.

Recently, Trouet et al (1,2) showed experimentally that daunorubicin[6] as well as adriamycin[7] acquire characteristics of lysosomotropic drugs when combined with DNA. Some of our observations regarding the DNA-adriamycin complex have been published elsewhere (3,4). This report deals with our latest results achieved in animals and man with the DNA-adriamycin complex.

MATERIALS AND METHODS

Solutions of the DNA-adriamycin(T) complex[8] were prepared as previously described (1). Fifty ml of the DNA solution containing 4.68 mg/ml was added to 1 ml of the adriamycin solution containing 20 mg/ml.

Studies in Mice

Experimental Animals and Tumors

BDF_1 (DBA/2 × C57BL) and DBA/2 mice supplied by the Charles River Laboratory were used. In the present work, an L1210 leukemia ascites form was used for inoculation and was maintained by serial propagation in DBA/2 mice in our laboratories. One line of this leukemia was originally obtained from Mr. I. Wodinsky (Arthur D. Little, Inc.) and one was obtained from Dr. A. Trouet. In this report, these lines are identified either by the label L1210(W) (Wodinsky) or L1210(T) (Trouet).

Experimental Design

A total of 10^5 L1210(W) or L1210(T) leukemic cells in 0.1 ml of Hank's balanced solution were injected

[1]Supported by grant No. 20187 from the Fonds de la Recherche Scientifique Médicale, Belgium.

[2]DNA-adriamycin: nucleic acid, deoxyribo-, sodium salt, compd. with (8*S-cis*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride.

[3]Département de Chimiotherapie (Drs. Rozencweig, Kenis, and Staquet) and Section de Chimiothérapie Expérimentale (Drs. Atassi and Duarte-Karim), Service de Médecine Interne et Laboratoire d'Investigation Clinique, Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, Belgium.

[4]We wish to express our gratitude to Dr. A. Trouet for supplying the DNA and the DNA-adriamycin(T), and to Dr. J. Longueville for providing his clinical data concerning the DNA-adriamycin(T).

[5]*Reprint requests to:* Dr. M. Rozencweig, Departement de Chimiothérapie, Service de Médecine Interne et Laboratoire d'Investigation Clinique, Institut Jules Bordet, Centre des Tumeurs de l'Universite Libre de Bruxelles, Brussels, Belgium.

[6]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

[7]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8. Supplied by Farmitalia Benelux.

[8]DNA-adriamycin(T) indicates the complex prepared according to the method of the "Laboratoire de Chimie Physiologique de l'Université Catholique de Louvain," Belgium. For studies in mice, this complex was prepared in our laboratories and the DNA (DNA type V from Sigma Chemical Corp., St. Louis, Mo) was supplied by Dr. A. Trouet. For studies in men, DNA-adriamycin(T) was supplied by Dr. A. Trouet. NSC-169534 indicates the lyophilized solution of the DNA-adriamycin complex prepared at the National Cancer Institute, Bethesda, Md.

intravenously (iv) into BDF_1 and DBA/2 male mice (23-25 g) in the first experiment. Twelve animals were used for each test. The increase in lifespan (ILS) as well as the body weight change on Day 5 (BWC) after adriamycin and DNA-adriamycin(T) administration, were then recorded. These parameters indicated the therapeutic effectiveness and the drug-induced toxic effects.

In another experiment, 10^5 L1210(T) leukemic cells were inoculated iv or subcutaneously (sc) on the inner side of the right thigh of DBA/2 male mice (17-18 g) in order to compare the effectiveness of adriamycin, DNA-adriamycin(T), and NSC-169534 against the solid and blood forms of L1210 leukemia. Endpoints considered included both ILS and measurements of tumor size on Day 9 (TS).

In both experiments, treatment began 24 hours after inoculation of L1210 cells and continued for 5 consecutive days at a dose of 6 mg of free or bound adriamycin/kg.

In a third experiment, the plasma levels of adriamycin, DNA-adriamycin(T), and NSC-169534 were determined at selected intervals after iv inoculation of these drugs at a dose of 6 mg/kg of free or bound adriamycin to DBA/2 male mice (~25 g) bearing L1210(T) leukemia (10^5 cells/mouse). Venous blood samples were collected from the inferior vena cava under open thoracotomy of the anesthetized animal. The blood from five or six mice was pooled and the plasma was separated immediately and stored at -20°C for a maximum of 1 month before drug determination.

Studies in Man

Phase I Trial

A phase I trial with DNA-adriamycin(T) was undertaken in cooperation with Dr. J. Longueville of the Université Catholique de Louvain (Belgium). A total of 19 patients with different types of advanced solid tumors not suitable for surgery or radiotherapy were selected for this study. Most of the patients had been treated previously with other chemotherapeutic agents. The drug was usually administered as a 6-12-hour infusion in a dose schedule of 75 mg/m^2 every 3 weeks, similar to the dose schedule already widely recommended for free adriamycin (5-7). Fever and chills occurred with shorter infusions. One to five courses of DNA-adriamycin(T) were given but the majority of the patients did not receive more than two courses.

Plasma Levels of Adriamycin

Free adriamycin, DNA-adriamycin(T), and the lyophilized NSC-169534 compound were given in iv infusions lasting from 1.15 to 4.30 hours to different groups of four or five patients with cancer at a dose of 75 mg/m^2 of free or bound adriamycin. Bilirubin was always found to be within the normal range whereas borderline urea and creatinine serum levels were occasionally encountered. Ten-ml samples of heparinized venous blood were collected before drug administration and at specified intervals thereafter. The samples were centrifuged immediately and the plasma was separated, frozen, and stored at -20°C for a maximum of 1 month before drug determination.

Total Plasma Fluorescence Assay

Total adriamycin fluorescence was assayed by a modification of the method of Bachur et al (8) using a Zeiss PMQ3 spectrophotofluorometer and a Beckman Spinco L265B ultracentrifuge. The method used for adriamycin as well as for the DNA-adriamycin complexes is extensively reported elsewhere.[9]

RESULTS

Studies in Mice

Table 1 summarizes the difference in the therapeutic effectiveness and the toxic effects of adriamycin and the DNA-adriamycin(T) complex. The latter compound was more active than free adriamycin, at the particular dose schedule of 6 mg/kg of free or bound adriamycin given daily for 5 consecutive days to DBA/2 mice inoculated iv with L1210(T) leukemia cells. This difference in therapeutic effectiveness was less apparent when L1210(W) leukemia was inoculated in the same strain of mice, and the difference disappeared completely when L1210(T) or L1210(B) was inoculated in BDF_1 mice.

The enhanced therapeutic effect of DNA-adriamycin(T) over adriamycin alone in DBA/2 mice inoculated iv with L1210(T) cells completely disappeared when the same mice were inoculated sc with the same cancer cells (solid form). Furthermore, free adriamycin given iv appeared to be more active than DNA-adriamycin(T) given iv in preventing the development of the primary tumor despite higher cumulative toxic effects as indicated by the body weight change (table 2).

NSC-169534 did not show the same increased therapeutic effectiveness as DNA-adriamycin(T) against L1210(T) cells inoculated iv. The inhibitory effect of NSC-169534 on the primary tumor was less significant than that observed with either adriamycin or DNA-adriamycin(T) solutions used under the same conditions.

Preliminary results of our pharmacologic studies in mice with these compounds are presently available. Figure 3 shows the mean plasma values of total

[9]Staquet M, Rozencweig M, Duarte-Karim M, et al. Pharmacokinetics of adriamycin and DNA-adriamycin in L1210 mice and in man. Submitted for publication.

drug fluorescence following a single iv direct administration of either of the three compounds. Free adriamycin underwent a short distribution phase followed by a relatively sustained excretion phase. This plasma disappearance curve is very similar to that obtained after injection of NSC-169534. On the contrary, the mean curve observed after DNA-adriamycin(T) is completely different with regard to initial values as well as to the pattern of plasma disappearance. DNA-adriamycin(T) initial values are about 50 times higher than the initial adriamycin values. Moreover, the DNA-adriamycin(T) complex is cleared from the plasma according to a single linear phase, at least within our experimental period. Ninety minutes after the administration of the complex, the mean fluorescence value still remained higher than the mean value measured after the free drug administration.

TABLE 1.—Comparison of the effect of adriamycin and DNA-adriamycin(T) on the lifespan of DBA/2 and BDF_1 mice with L1210 leukemia*

	L1210 (T)				L1210 (W)			
	Adriamycin		DNA-adriamycin (T)		Adriamycin		DNA-adriamycin (T)	
	ILS (%)	BWC (g)	ILS (%)	BWC (g)	ILS (%)	BWC (g)	ILS (%)	BWC (g)
DBA/2 mice	83	-3.25	216	-2.7	0	-2.5	83	-2.5
BDF_1 mice	0	-5.5	6	-3.3	0	-3.1	6	-1.6

*DBA/2 and BDF_1 male mice (23-25 g) were inoculated iv on Day 0 with 10^5 leukemic cells from stock DBA/2 mice bearing ascitic L1210. Twelve mice were used per test. Daily treatment was given from Days 1 to 5. ILS = increase in lifespan; BWC = body weight change on Day 5.

TABLE 2.—Comparison of the effect of adriamycin and DNA-adriamycin complexes on the lifespan of DBA/2 mice with L1210(T) leukemia*

	Adriamycin			DNA-adriamycin complexes						Control		
				DNA-Adriamycin(T)			NSC-169534					
	ILS (%)	BWC (g)	TS (mm^3)	ILS (%)	BWC (g)	TS (mm^3)	ILS (%)	BWC (g)	TS (mm^3)	ILS (%)	BWC (g)	TS (mm^3)
L1210(T) iv	1	-3.0		85	-1.1		41	-1.5		0	+0.5	
L1210(T) sc	89	-4.3	6.9	62	-2.8	34.6	59	-1.7	66.8	0	+0.6	597.3

*DBA/2 male mice (17-18 g) were inoculated iv or sc on Day 0 with 10^6 leukemic cells from stock DBA/2 mice with ascitic L1210 leukemia. Ten mice were used per test. Daily treatment was given from Days 1 to 5. ILS = increase in lifespan; BWC = body weight change on Day 5; TS = tumor size on Day 9.

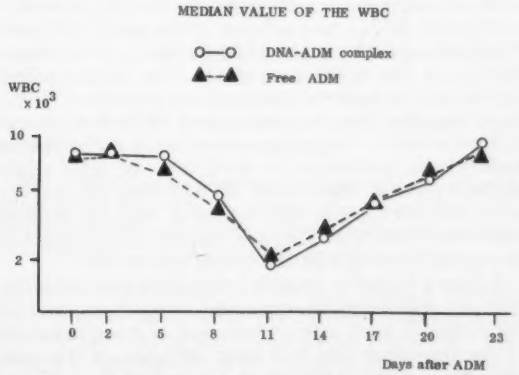

FIGURE 1.—Median value of the wbc count after 6-12-hr infusion of the DNA-adriamycin(T) complex (DNA-ADM) and direct injection of free adriamycin (free ADM). Bound and free adriamycin were given at a dose of 75 mg/m^2 to 12 and 60 patients respectively.

Studies in Man

Phase I Trial

Antitumor effect could not be evaluated accurately since most patients had highly advanced disease and had proven resistance to prior chemotherapy. However, three significant objective responses were obtained with a short remission duration ranging from 2 to 3.5 months: one in Ewing's sarcoma, one in small cell carcinoma of the lung, and one in squamous cell carcinoma of the cervix.

Subacute clinical as well as hematologic toxic effects are clearly not reduced when using infusions of DNA-adriamycin(T) as compared to iv push injection of the free compound given at the same dose schedule (table 3, figs 1 and 2).

TABLE 3.—Clinical toxic effects of adriamycin and DNA-adriamycin(T) complex*

Toxic effects	Percent occurrence after administration of— Adriamycin (50 patients)	DNA-adriamycin(T) complex (12 patients)
Alopecia	94	100
Nausea/vomiting	50	50
Diarrhea	6	8
Stomatitis	4	30

*Data obtained from Kenis et al, 1973.

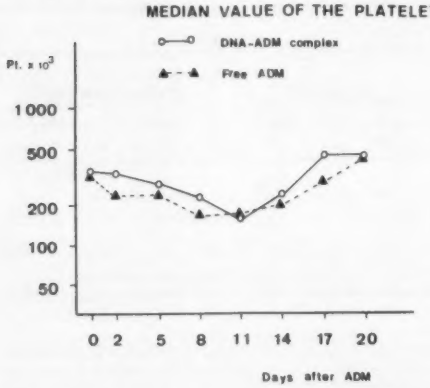

FIGURE 2.—Median value of the platelet count after 6-12-hr infusion of the DNA-adriamycin(T) complex (DNA-ADM) and direct injection of free adriamycin (free ADM). Bound and free adriamycin were given at a dose of 75 mg/m² to 12 and 60 patients respectively.

Plasma Levels of Free and Bound Adriamycin

The mean plasma values of total drug fluorescence were determined in patients after a 75-470-minute infusion of adriamycin, DNA-adriamycin(T), and NSC-169534 (fig 4). In this experiment, the three compounds displayed a biphasic plasma disappearance. After free adriamycin administration, the mean calculated biologic half-lives for the first and the second part of the curve were 0.37 and 31.5 hours respectively. As previously demonstrated in mice, there is obviously no marked difference between NSC-169534 and the free adriamycin mean curve. As in the mouse experiments, the initial values obtained after the DNA-adriamycin(T) administration were much higher than those observed after free adriamycin, despite the fact that on an average, the complex was given in a twofold longer infusion than the free drug. After DNA-adriamycin(T) administration, the mean calculated biologic half-lives for the first and the second part of the mean total fluorescence curve were 0.32 and 2.77 hours respectively.

DISCUSSION

In animal models, the therapeutic action of the DNA-adriamycin(T) complex appears to require intimate contact between the complex and the tumor

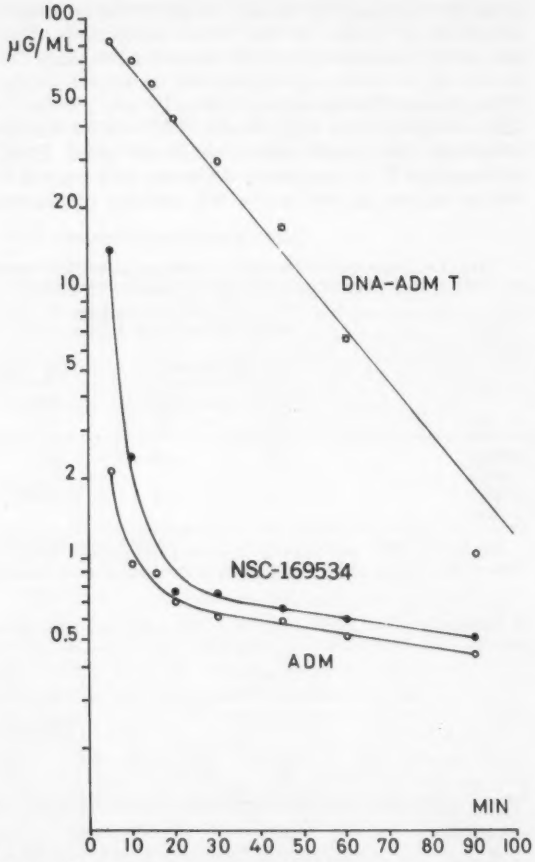

FIGURE 3.—Total plasma fluorescence disappearance mean curves after iv administration of 6 mg/kg of free or bound adriamycin to DBA/2 mice. Two DNA-adriamycin complexes were used: DNA-ADM(T) and NSC-169534.

cells, as suggested by the opposite results in experiments with L1210 as a solid sc tumor and L1210 as a true leukemia. Moreover, the previously shown superiority of the complex over the free drug against L1210 murine leukemia depends on the line of L1210 cells and the strain of mice used. Different plasma DNAse activity in each strain of mice and different endocytosis properties in both lines of cells might explain some of these data. In addition, this superiority in therapeutic effectiveness may be reduced when the toxicity of free adriamycin is decreased by changing the schedule of administration (4).

A phase I trial in man failed to demonstrate a significant difference in subacute clinical as well as hematologic toxic effects between iv push injection of the free drug and 6-12-hour infusion of the complex, both given at the same dose schedule of 75 mg/m² every 3 weeks. However, in this small series, total doses of the complex were too low to provide accurate evaluation of cardiac toxicity since this side

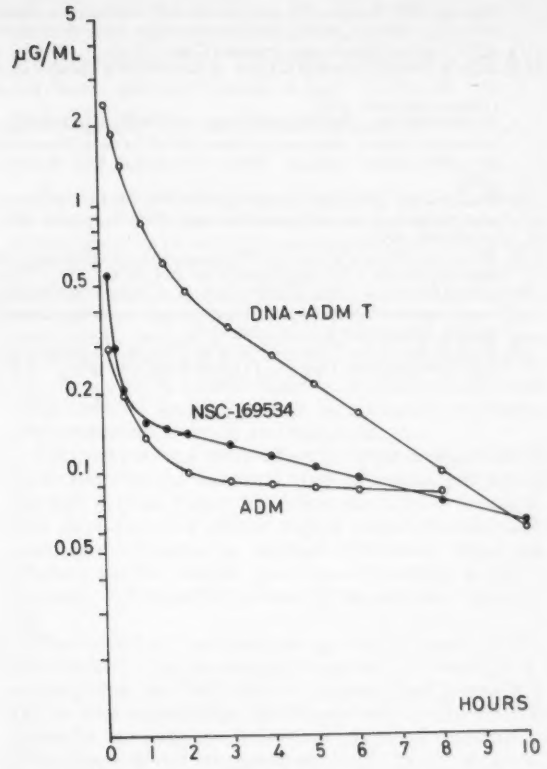

FIGURE 4.—Total plasma fluorescence disappearance mean curves after iv administration of 75 mg/kg of free or bound adriamycin to groups of 4 or 5 patients. Drugs were given as a 1.15-4.30-hr infusion. Two DNA-adriamycin complexes were used: DNA-ADM(T) and NSC-169534.

effect is obviously dose related when the free compound is used. More extensive clinical trials are needed to confirm the hypothesis that cardiac toxicity is reduced with the complex as compared to the free drug. Lyophilized solutions would be highly desirable in this regard, since the solutions presently available are still home-made and are prepared shortly before administration. However, the problems in large-scale preparation of such standardized compound have not yet been solved.

Our preliminary pharmacokinetic study clearly demonstrates in mice as well as in man striking differences between free and bound plasma adriamycin levels measured by a total fluorescence assay.

In mice, initial levels and concentration × time (c × t) are much higher after the complex is given than after the free drug administration. Moreover, the complex is cleared from the plasma according to a single linear phase whereas the free drug displays a biphasic plasma disappearance curve at least within our experimental period.

In man, despite the fact that initial levels and c × t are also much higher after the complex is given than after the free drug administration, there are similar clinical and hematologic subacute toxic effects with both forms of the drug given at the same dosage. These findings would therefore suggest that the complex could achieve the same therapeutic effectiveness as the free drug with lower doses and consequently a reduced toxicity, provided that initial levels and c × t have a therapeutic significance. However, it remains to be seen whether both forms of the drug induce the same toxicity when given as a direct injection or as an infusion.

Adriamycin and DNA-adriamycin(T) are cleared from the plasma according to a biphasic curve for both forms of the drug: the value of the half-life calculated for the first part of that curve is within the range of values reported by other investigators using free adriamycin (9,10). However, the half-life of free adriamycin calculated for the slow part of the curve in our investigation as well as in the experiments of Benjamin et al (9) and Wilkinson et al (10) is markedly longer than that found for DNA-adriamycin(T). The complex is theoretically less diffusable than the free drug and might therefore be less rapidly catabolized. Benjamin et al (6) have found that half-lives during the slow phase of the plasma fluorescence disappearance are about twice as long for the adriamycin metabolites than for adriamycin alone. This observation would only partially explain our data since Benjamin et al reported that the mean half-life for true adriamycin fluorescence was 16.7 hours, about seven times greater than that found for the bound drug. Moreover, other investigators did not find adriamycin metabolites in either mice or man (11,12).

CONCLUSION

It has been shown experimentally that adriamycin may acquire lysosomotropic characteristics when combined with DNA. However, a more specific cytotoxic activity with such a complex remains speculative.

The previously assessed superiority of the DNA-adriamycin complex over adriamycin alone in L1210 leukemia is reproducible only under very particular experimental conditions concerning the strain of mice, the line of L1210 leukemia, and the route of tumor cell inoculation.

Further investigation is needed to compare accurately the therapeutic index of free and bound adriamycin especially with regard to drug-induced cardiac side effects.

In mice as well as in man, initial plasma levels were higher with the complex than with the free drug, and striking differences in the pattern of clearance from the plasma were observed. No firm conclusion can be drawn from these data.

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Experimental Tumor Activity of Adriamycin (NSC-123127)[1]

Abraham Goldin and Randall K. Johnson[2,3]

The clinical interest in adriamycin, the 14-hydroxy derivative of daunorubicin, stems from preclinical investigations in which it was demonstrated to exert therapeutic activity against a wide variety of experimental tumors in animals. Table 1 lists the experimental tumor systems in which adriamycin has demonstrated therapeutic activity; these results are based on the studies of a number of investigators (1-14). Responsive tumors include transplantable and viral-induced leukemias and lymphomas, myeloma, sarcomas, carcinomas, and melanoma.

Adriamycin was more effective than daunorubicin in increasing the survival time of animals bearing Ehrlich ascites tumor (1). When given for 5 consecutive days starting on the day of tumor inoculation, adriamycin exerted a marked inhibitory effect on Ehrlich ascites tumor growth and yielded a 187% increase in the survival time of the animals (table 2) (1).

The activity of adriamycin against a transplantable ascites lymphosarcoma induced by 1-methyl-1-nitrosourea in C3Hf mice is summarized in table 3 (1). In this experiment the tumor was in the fourth transfer generation following chemical induction. Treatment given intravenously (iv) for 8 consecutive days from the day after tumor inoculation with a dose of 2.5 mg/kg/day resulted in a 93.4% increase in the survival time of the animals. With this tumor also, adriamycin given in equitoxic doses was more effective than daunorubicin.

In the experiments of Di Marco et al (9) involving adriamycin treatment of subcutaneously (sc) transplanted C3H mammary carcinoma, the tumors were derived from spontaneous carcinoma and were in the third to the sixth transplant generation. An experiment summarizing the activity of adriamycin given iv against transplanted C3H mammary carcinoma is shown in table 4 (9). In this experiment the iv treatments were administered every other day from Days 1 to 11 and from Days 17 to 27 after tumor inoculation. Using this iv modality, marked inhibition of tumor growth was obtained, accompanied by a significant increase in the survival time of the animals. With a dose of 2.5 mg/kg there were eight survivors among ten animals at the end of the experiment (210 days) as compared with no survivors among ten for the untreated controls. In this study it was also determined that adriamycin was more effective than daunorubicin in the treatment of C3H mammary carcinoma.

In another study involving treatment of first-generation transplants of spontaneous mammary tumors of C3H/He mice by Sandberg and Goldin (9), adriamycin was ineffective in inhibiting tumor growth or in increasing the median survival time of the animals. In these experiments adriamycin was given intraperitoneally (ip) every 3 hours for a single day. The treatment was given at a time when the tumor was more advanced (4-6 mm in diameter). It is not clear to what extent the difference in scheduling of drug administration or the advancement of the tumor contributed to the loss of effectiveness. In this connection we should mention the report of Lenaz and Di Marco (16) in which the authors caution that the route of administration of adriamycin may be important in the determination of therapeutic outcome. When administered ip both daunorubicin and adriamycin may induce peritonitis and peritoneal adhesions, resulting in increased toxicity as compared with the iv or sc route. It is their suggestion that for the treatment of solid tumors the iv route of administration (or where not possible the sc route) is preferable. On the other hand, the ip treatment of ascitic tumors, such as leukemia L1210, may yield better results than iv treatment since ip treatment subjects the tumor cells to an initial high concentration of drug.

Schabel (8) has conducted extensive studies with the Ridgway osteogenic sarcoma in which the response to therapy is determined by treatment of animals with a large palpable tumor mass. The response to therapy is followed carefully in the individual animals. In these studies the response parameters for the individual mice include: *(a)* extent of regression of palpable tumor, with classification as to partial or complete regression; *(b)* the median duration of complete regression; *(c)* the number, rate of growth, and time of death of animals in which tumor relapse occurs; and (*d*) the number of cures.

The influence of actinomycin D, adriamycin, cyclophosphamide, and melphalan on the regression of established Ridgway osteogenic sarcoma is summarized in table 5 (8). The four drugs, administered as single treatments, were highly effective in producing complete regressions of the Ridgway osteogenic sarcoma. Of the four drugs, actinomycin D was most effective, yielding eight complete regressions and seven "cures" among eight animals. The marked chemotherapeutic activity of actinomycin D against

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[1]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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TABLE 1.—Experimental tumor systems in which adriamycin has demonstrated therapeutic activity

Tumor system	Investigators	Year	Ref No.
Lymphoma, leukemia			
Lymphosarcoma, ascites	Di Marco et al	1969	1
Leukemia L1210	Sandberg et al	1970	2
Leukemia P388	Sandberg et al	1970	2
Leukemia P288	Schwartz and Grindey	1973	3
Gross leukemia	Di Marco et al	1973	4
AKR lymphoma, spontaneous	Frei et al	1974	5
Myeloma			
Oberling-Guérin-Guérin myeloma	Di Marco et al	1969	1
Sarcoma			
Sarcoma 180	Di Marco et al	1969	1
Murine rhabdomyosarcoma (Moloney virus-induced, primary)	Casazza et al	1971	6
Yoshida sarcoma	Hoshino	1972	7
Ridgway osteogenic sarcoma	Schabel	In press	8
Carcinoma			
Ehrlich ascites carcinoma	Di Marco et al	1969	1
C3H mammary carcinoma (spontaneous, early transplant generations)	Di Marco et al Griswold et al	1972 1973	9 10
Walker 256 carcinosarcoma	Goldin and Johnson	1974	11
BALB/3T3 (Kirsten murine sarcoma viral-transformed, nonproducing)	Keys et al	1974	12
Lewis lung carcinoma (iv)	Ovejera et al	1975	13*
Melanoma			
B16 melanoma (ip)	Goldin and Johnson	1974	14

**See also* footnote 4.

TABLE 2.—Effect of adriamycin on Ehrlich ascites carcinoma*

	Dose† (mg/kg/day)	Average survival time (days)	ILS (%)
Adriamycin	2.50	51.2	187
	1.25	31.8	78
Controls	—	17.8	

*Adapted from Di Marco et al (1).
†Treatment ip for 5 consecutive days from day of tumor inoculation.

TABLE 3.—Effect of adriamycin on a transplantable lymphosarcoma (ascites) in C3H mice*†

Adriamycin dose‡ (mg/kg/day)	Average survival time (days)	ILS (%)
2.5	38.1	93.4
2.0	30.3	52.8
Controls	19.7	—

*In 4th generation after induction by 1-methyl-1-nitrosourea. 10^6 ascites cells inoculated ip in male and female mice.
†Data of Di Marco et al (1).
‡Treatment iv for 8 consecutive days from day after tumor inoculation.

TABLE 4.—Effect of adriamycin on transplanted C3H mammary carcinoma*

Adriamycin dose† (mg/kg/day)	Average survival time (days)	No. of survivors (210 days)/ total No. treated
2.5	190.5	8/10
2.0	174.7	4/10
Controls	90.5	0/10

*2×10^7 tumor cells inoculated sc in male and female C3H mice. Adapted from Di Marco et al (9).
†Treatment iv every other day, Days 1-11, 17-27.

early or advanced Ridgway osteogenic sarcoma has also been reported by other investigators (17,18). Treatment with adriamycin resulted in seven complete regressions among eight animals, but tumors recurred and none of the animals were "cured."

Although daunorubicin also exerted therapeutic activity against Ridgway osteogenic sarcoma, it did not appear to be as effective as adriamycin in inducing complete regressions (8).

The toxicity of adriamycin administered ip to normal BDF_1 mice on various schedules of administration is summarized in table 6. LD10 per injection, the total LD10, and the total LD10 relative to the LD10 for a single treatment (Day 1 only) were determined for a variety of treatment schedules. The data indicate that the toxicity of adriamycin on successive doses is essentially additive. Even with an extensive interval between treatment (Days 1 and 15; Days 1 and 21), there was not a marked increase in the total dose relative to treatment on Day 1 only. Treatment every 3 hours on Day 1 only appeared to result in greater-than-additive toxicity relative to a single dose (Day 1 only), but this was not reflected on treatment given every 3 hours on Days 1, 5, and 9. For the various schedules the slopes of the dose-mortality curves varied, the ratios of LD90 to LD10 ranging from 1.22 to 3.29 (table 6). Typical dose-response data have been reported in previous publications (6,19,20). Adriamycin does not appear to be extensively metabolized in vivo (21,22) and there is drug retention in tissues after treatment of mice (21). These results are consistent with the cumulative toxicity observed even with intermittent dosage schedules.

Adriamycin has been reported to be more effective than daunorubicin in the treatment of leukemia L1210 over a wide range of schedules of drug administration (2, 14). In earlier studies it had been reported that multiple ip treatment with adriamycin on a single day was the optimal schedule for leukemia L1210 (2). However, a compilation of the data of numerous recent experiments on treatment of early leukemia L1210, summarized in table 7, indicates that adriamycin has no definite schedule dependency in this tumor system. Although in some experiments multiple treatment (every 3 hours) for a single day was optimal, this observation was not consistently reproducible.

With the various schedules of treatment for the leukemic mice, consistent with toxicity studies in nonleukemic mice, the optimal therapeutic total dose did not vary markedly and on most schedules was somewhat less than the LD10 for normal mice. However, for the intermittent schedule (every 3 hours on Days 1 and 9) the total optimal dose (20.8 mg/kg) was approximately twice that observed for a single treatment (9.8 mg/kg). Although this schedule was not more effective than single treatment or treatment every 3 hours on Day 1 only, it is suggested that intermittent scheduling is worthy of further investigation. The lack of reproducibility of the every 3 hour schedule on Day 1 only could be due to the fact that optimal therapeutic effects were obtained at dose levels higher than the LD10.

The effect of drug route on adriamycin activity against ip inoculated leukemia L1210 is summarized in table 8 (2,23). Adriamycin activity against leukemia L1210 appears to be route-dependent. The drug was active on both ip and sc administration but was inactive on oral administration. The decreased activity on oral administration suggests a lack of absorption of the drug from the gastrointestinal tract. In another experiment (23) it was observed that iv administration of adriamycin was also effective against ip inoculated leukemia L1210. However, there appeared to be some reduction in therapeutic effectiveness with the iv route of drug administration as compared with the ip route.

Treatment with adriamycin was ineffective in increasing the survival time of mice with intracranially inoculated leukemia L1210 cells. This observation is consistent with evidence that adriamycin is incapable of crossing the blood-brain barrier (24).

The influence of schedule of administration and also of tumor inoculum level on the activity of adriamycin against ip inoculated P388 leukemia is summarized in table 9. Adriamycin is highly effective in the treatment of ip inoculated P388 leukemia. At inoculum levels of 10^5 and 10^6 cells, on four schedules of treatment, extensive increases in survival time and a high percentage of survivors were obtained. At a tumor inoculum level of 10^7 cells the therapeutic effectiveness of adriamycin was somewhat reduced, but no definite schedule dependency was observed.

The influence of route of adriamycin administration and of stage of disease advancement on activity against ip inoculated P388 leukemia is summarized in table 10. Although both ip and iv treatment produced marked increases in survival time against ip inoculated P388 leukemia, the ip route of treatment was more effective. Using a single treatment 1 day after leukemic inoculation, ip administration resulted in survival of all ten of the animals, whereas with the iv route there was a 212% increase in survival time and three of ten animals survived.

The stage of disease advancement when therapy is started may have a profound influence on the therapeutic effectiveness of a drug. It is well established that the therapeutic efficacy of a drug is decreased as the body burden of tumor cells is increased. In the treatment of ip P388 leukemia (table 10), both by the ip and iv route of adriamycin administration, there was a reduction in antileukemic effectiveness when treatment was withheld until the fifth day after leukemic inoculation. With treatment on the fifth day, however, it is again indicated that treatment by the ip route was more effective than treatment by the iv route.

The influence of schedule of administration and stage of disease advancement on the activity of adriamycin against ip B16 melanotic melanoma is summarized in table 11. In the treatment of ip B16

TABLE 5.—Treatment of established Ridgway osteogenic sarcoma (treatment ip on Day 12 after implant)*

Drug	Dose (mg/kg)	No. of regressions†/ No. of animals treated		Median duration of CR‡ (days)	No. of "cures"/ No. of animals treated
		Partial	Complete		
Actinomycin D	0.6	—	8/8	—	7/8
Adriamycin	14.0	—	7/8	11	0/8
Cyclophosphamide	15.6	—	8/8	21	3/8
Melphalan	10	1/8	7/8	28	4/8

*Adapted from Schabel (8).
†Partial = >50% reduction in original tumor mass; complete = regression to below palpable size (<65 mg).
‡In animals that relapsed following complete remission.

TABLE 6.—Toxic levels of adriamycin on various schedules of administration (ip)

Dose schedule	LD10* (mg/kg)		LD90/LD10	Ratio of total dose to Day 1 only dose
	Per injection	Total dose		
Day 1 only	13.2	13.2	2.56	1.00
Daily Days 1-7	2.8	19.6	1.54	1.48
Daily Days 1-9	1.7	15.3	2.06	1.16
Days 1,3	7.3	14.6	1.22	1.11
Days 1,5	5.9	11.8	1.44	0.89
Days 1,15	8.6	17.2	1.98	1.30
Days 1,21	10.0	20.0	2.60	1.52
Days 1,5,9	4.4	13.2	1.80	1.00
q3h Day 1 only	0.7	5.6	3.29	0.42
q3h Days 1,5,9	0.7	16.8	2.43	1.27

*LD10 values were determined from dose-mortality regression lines in normal BDF_1 mice.

TABLE 7.—Effect of treatment schedule on the antileukemic (L1210) effectiveness of adriamycin*

Dosage schedule	Optimal dose (mg/kg)		ILS† (%)	S/T‡
	Per injection	Total dose		
Day 1 only	9.8	9.8	63	1/12
Daily Days 1-5	1.2	6.0	45	1/12
Daily Days 1-9	1.3	11.7	43	0/10
Days 1,5,9	3.6	10.8	39	0/10
Days 1,9	7.0	14.0	38	0/10
q3h Day 1 only	1.5	12.0	61	1/10
q3h Days 1,5,9	0.6	14.4	41	0/10
q3h Days 1,9	1.3	20.8	63	1/12

*Average values from 3-25 dose-response experiments for the various dosage schedules.
†ILS = increase in median survival time over untreated controls.
‡S/T = survivors over total at Day 60.

TABLE 8.—Effect of drug route on adriamycin activity in leukemia L1210 inoculated ip*

Drug route	Schedule (days)	Optimal dose (mg/kg/day)	ILS† (%)
ip	1-5, 11-15, 21-25	2.0	63
sc	1-5, 11-15, 21-25	2.0	54
oral	1-5, 11-15, 21-25	8.0	4

*BDF_1 male mice (18-26 g); ip inoculum = 10^5 cells. Adapted from Goldin (23).
†ILS = increase in median survival time over untreated controls.

melanoma there was no evidence of schedule dependency. When treatment was initiated 1 day after ip inoculation of the tumor, adriamycin was highly effective in producing long-term survivors on the four schedules of treatment employed. On all four schedules there was an approximately equivalent reduction in therapeutic effectiveness when the initiation of treatment was delayed until the fifth day after tumor inoculation.

The Lewis lung carcinoma is an interesting model for investigation, since, after iv inoculation or as the result of metastatic spread from an sc or intramuscular (im) site, there is preferential growth of the tumor in the lungs (13,25). The activity of adriamycin against Lewis lung carcinoma implanted iv or sc is summarized in table 12 (13).[4] Over a series of dose levels adriamycin was more effective when administered by the iv route than by the ip route in extending the survival time of mice with iv inoculated Lewis lung carcinoma. When treatment was administered 1 day after tumor inoculation, a single 14-mg/kg treatment resulted in a 126% increase in survival time and two survivors among eight animals. When treatment was delayed until 7 days after tumor inoculation there was a reduction in therapeutic effectiveness, and at the optimal dose (14 mg/kg) the increase in survival time was 60% and there was one survivor among eight animals. When treatment was delayed until the 14th day, the therapeutic response was markedly reduced. On all of these schedules, however, iv administration of the drug was more effective than ip administration.

The type of model employed is important in determining the extent of therapeutic response obtained with a drug. This is illustrated in the treatment with adriamycin of sc inoculated Lewis lung carcinoma (table 12). With this tumor model, as opposed to the iv implanted tumor model, treatment administered iv or ip against early or advanced disease was ineffective. It may be noted, however, that in other studies with this sc model (14) some inhibition of local tumor growth was observed on treatment with adriamycin, but only at doses that were fairly toxic as indicated by the weight loss of the animals.

The importance of clinical staging in determining the response to adriamycin is well illustrated in studies by Schabel (8) with the Ridgway osteogenic sarcoma (table 13). A single treatment (15 mg/kg) administered sc 12 days after tumor implantation resulted in a high percentage of regressions (two partial and eight complete among ten animals) and a marked increase in the lifespan of the animals. When treatment with the same drug dose (15 mg/kg) was delayed until the 17th day after implant, there were only four partial regressions, no complete regressions, and no increase in lifespan among the ten animals. No cures were observed with treatment on either the 12th or the 17th day after tumor inoculation.

[4]Ovejera AA, Johnson RK, and Goldin A. Unpublished data.

Following adriamycin treatment over a series of transplant generations, a subline of P388 leukemia was established which is markedly resistant to the drug. Table 14 lists the drugs to which the subline remained sensitive and the drugs to which it exhibited cross-resistance. The retention of sensitivity to the established antitumor drugs cytosine arabinoside, methotrexate, 5-fluorouracil, cyclophosphamide, BCNU, and melphalan suggests that these drugs may be of interest for utilization following the development of any clinical resistance to adriamycin. The relatively broad range of cross-resistance is of interest. There was complete or partial cross-resistance to drugs with diverse mechanisms of action including mitotic inhibitors (vincristine and vinblastine), DNA binding agents (cinerubin A, ellipticine, and actinomycin D), and an inhibitor of protein biosynthesis (emetine). The adriamycin-vincristine cross-resistance appears to be reciprocal. Danø (20,26) observed that sublines of Ehrlich ascites tumor resistant to adriamycin or vincristine showed mutual cross-resistance. The cross-resistance to daunorubicin and to adriamycin-14-octanoate would be expected. It would be important to determine whether any other adriamycin analogs would show therapeutic activity against an adriamycin-resistant tumor subline. It should be noted in this connection that cinerubin A, which has some general structural similarity to adriamycin and is active against the sensitive P388 leukemia, did retain partial activity against the adriamycin-resistant subline.

Cross-resistance between adriamycin and daunorubicin has also been demonstrated in Ehrlich ascites carcinoma (20), leukemia L1210 (7), and Yoshida sarcoma (7). Sublines of leukemia L1210 resistant to 6-mercaptopurine or 5-fluorouracil retained sensitivity to adriamycin (7). A methotrexate-resistant subline of leukemia L1210 was collaterally sensitive to adriamycin (7). Sublines of Yoshida sarcoma resistant to cyclophosphamide, 5-fluorouracil, 6-mercaptopurine, or mitomycin C retained therapeutic responsiveness to adriamycin (7).

Adriamycin and daunorubicin have demonstrated immunodepressive properties (6,27-29). An experiment involving the effects of pretreatment with daunorubicin or adriamycin on the growth of the virus-induced primary Moloney sarcoma (table 15) is of interest in this connection. In this system, the Moloney sarcoma virus is inoculated im into weanling Swiss CD-1 mice. There is an initial period of progressive growth of sarcoma at the site of inoculation which is then followed by complete tumor regression. A series of pretreatments with daunorubicin (7.5 mg/kg/day) administered sc prevented tumor regression and the mice died with progressively growing tumors. This progressive growth of tumor following pretreatment suggests that daunorubicin is immunosuppressive. On the other hand, with pretreatment at equitoxic doses of adriamycin (5.2 mg/kg/day) the tumor grew and regressed in the same manner as in untreated controls, indicating that

TABLE 9.—Influence of schedule of administration and tumor inoculum level on the activity of adriamycin in P388 leukemia inoculated ip

Tumor inoculum level	Schedule of administration*							
	Day 1 only		q3h Day 1 only		Daily, Days 1-9		q4d Days 1,5,9	
	ILS (%)	S/T	ILS (%)	S/T	ILS (%)	S/T	ILS (%)	S/T
10^7	319	3/10	330	2/10	147	0/10	201	3/10
10^6	>400	6/10	>400	10/10	206	4/10	>400	7/10
10^5	>400	7/10	>400	9/10	>400	8/10	>400	7/10

*ILS = increase in median lifespan and S/T = survivors/total at an optimal dose from a dose-response study. Drug administered ip. Median survival time of untreated control BDF_1 mice: 10^7 cells, 9.3 days; 10^6 cells, 11.1 days; 10^5 cells, 14.1 days.

TABLE 10.—Influence of route of drug administration and stage of advancement of disease on the activity of adriamycin in P388 leukemia inoculated ip

Route of adriamycin administration	Schedule of administration*			
	Day 1 only		Day 5 only	
	ILS (%)	S/T	ILS (%)	S/T
ip	>400	10/10	98	0/10
iv	212	3/10	35	0/10

*ILS = increase in lifespan and S/T = survivors/total at an optimal dose from a dose-response study. 10^6 P388 cells inoculated ip in BDF_1 mice. Median survival time of untreated controls = 11.3 days.

TABLE 11.—Influence of schedule of administration and stage of advancement on the activity of adriamycin in B16 melanoma inoculated ip

Day of initiation of treatment	Schedule of administration*							
	Single day, 1 dose		Single day, q3h		Daily × 9		q4d·× 3	
	ILS (%)	S/T	ILS (%)	S/T	ILS (%)	S/T	ILS (%)	S/T
1	>200	7/10	>200	7/10	>200	8/10	>200	9/10
5	45	1/10	54	2/10	54	1/10	62	3/10

*ILS = increase in median lifespan and S/T = survivors/total at an optimal dose from a dose-response study. Drug administered ip. Median survival time of untreated control BDF_1 mice = 24.0 days.

TABLE 12.—Activity of adriamycin against Lewis lung carcinoma implanted iv or sc*

Tumor inoculated—	Single adriamycin dose (mg/kg)	% ILS (survivors/total) with treatment given—†					
		Day 1 only		Day 7 only		Day 14 only	
		iv	ip	iv	ip	iv	ip
iv‡	20	T	T	T	T	T	T
	14	126(2/8)	T	60(1/8)	17	26	7
	9.8	90(2/8)	45	26	22	24	19
	6.9	36	17	45(1/8)	22	26	5
	4.8	22	7	12	2	14(1/8)	9
sc§	14	T	T	T	0		
	9.8	T	T	6	T		
	6.9	14	17	2	14		
	4.8	0	0	0	0		
	3.4	0	0	6	4		

*Data of Ovejera (ref 13 and footnote 4).
†ILS = increase in lifespan; T = toxic.
‡10^5 viable cells implanted iv in groups of 8 BDF_1 mice; median survival time of untreated controls = 21.0 days.
§10^5 viable cells implanted sc in groups of 8 BDF_1 mice; median survival time of untreated controls = 32.0 days.

TABLE 13.—Ridgway osteogenic sarcoma: clinical staging and response to adriamycin*

Adriamycin dose (mg/kg, sc)	Treatment schedule, days postimplant	No. of regressions†/ No. treated		Median lifespan (days)	ILS (%)	No. of "cures"/ No. treated
		Partial	Complete			
15	12	2/10	8/10	62.0	153	0/10
15	17	4/10	0/10	40.0	12	0/10

*Adapted from Schabel (8).
†Partial = >50% reduction in original tumor mass; complete = regression to below palpable size (< 65 mg).

TABLE 14.—Sensitivity to a spectrum of antineoplastic agents of a subline of P388 leukemia resistant to adriamycin

Drugs to which resistant line retains sensitivity	Drugs to which resistant line exhibits cross-resistance
Cytosine arabinoside	Daunorubicin
Methotrexate	Adriamycin-14-octanoate
5-Fluorouracil	Vincristine
Cyclophosphamide	Vinblastine
BCNU	Ellipticine
Melphalan	Emetine
Neocarzinostatin	Actinomycin D (partial)
	Cinerubin A (partial)

adriamycin is less immunosuppressive than daunorubicin.

Schwartz and Grindey (3) observed that adriamycin was more effective than daunorubicin in increasing the survival time of DBA/2 mice bearing ascitic P288 lymphocytic leukemia. However, the therapeutic advantage for adriamycin over daunorubicin was lost when animals were immunosuppressed by irradiation or cyclophosphamide prior to tumor inoculation. It was also observed that daunorubicin reached higher concentrations in the spleen than did adriamycin. The authors attributed the difference in therapeutic activity of these two drugs to a lower immunosuppressive activity for adriamycin.

Casazza et al (29) have reported that humoral antibody production against Moloney sarcoma virus was markedly inhibited by both daunorubicin and adriamycin. However, there was a difference in the effect on skin allograft survival time. Pretreatment with daunorubicin significantly increased skin allograft survival time while, at equitoxic doses of adriamycin, rejection occurred more rapidly. Thus, it is suggested that cell-mediated immunity is more extensively suppressed by daunorubicin than by adriamycin.

Adriamycin has been demonstrated to exert therapeutic synergism when employed in combination with a variety of known antitumor agents including alkylating agents, nitrosourea derivatives, plant alkaloids, antimetabolites, and others (table 16). The interest in using combination chemotherapy with adriamycin in the clinic is firmly supported by results in animal tumor systems (7,8,10,14,30). The combination of adriamycin with other drugs may result in improved therapeutic response as compared with the drugs employed individually. There is also the possibility of avoiding undesirable toxicity, particularly cardiomyopathy, by reduced long-term adriamycin dosage in combination. Use of combination chemotherapy may result in a delay in the origin of tumor cell resistance. The relationship of results in animal models to clinical effectiveness is a subject of considerable importance. One example of an investigation to determine correspondence of activity pertains to chemotherapy of breast cancer. Of the drugs active individually against breast cancer, cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, vinblastine, thioTEPA, and melphalan were therapeutically synergistic in combination with adriamycin in one or more experimental tumor systems. It will be of great interest to determine the carry-over of these observations to the clinic. It is only in this way that the applicability of tumor models for projection of clinical activity of two-drug combinations and polychemotherapy can be determined.

TABLE 15.—Effect of pretreatment with daunorubicin or adriamycin on viral-induced Moloney sarcoma*

Drug	Pretreatment dose on Days -3,-2,-1 before virus inoculation† (mg/kg/injection)	Tumor size on days—‡			No. of deaths with progressive tumor growth/No. treated
		8	15	30	
Daunorubicin	7.5	2.8	3.7	3.7	11/14
Adriamycin	5.2	1.8	2.9	1.2	2/16
Control	—	3.7	2.7	0.3	1/18

*Tumor inoculated im in 16-day-old CD-1 mice. Adapted from Casazza et al (6).
†Treatment sc at equitoxic doses.
‡Tumor size on an arbitrary scale of 1 through 5.

TABLE 16.—Experimental studies of adriamycin in combination with other antineoplastic agents

Adriamycin in combination with—	Activity in experimental tumor systems*						
	L1210	P388	B16	LL	AKR	C3H	ROS
Cyclophosphamide	+	+	+			+	+
Melphalan	−	+					
ThioTEPA	±						
BCNU	−						
CCNU	+						
Methyl-CCNU	−	+	+		−	+	
Vincristine	+	+					
Vinblastine	±						
Methotrexate	+	+					
5-Fluorouracil	−	+			−		
Ftorafur	−						
Cytosine arabinoside	+	+					
Cyclocytidine	+	+					
PalmO-ara-C		+					
5-Azacytidine	+	+					
NSC-45388	+		+	+	+	+	−
ICRF-159	+						
Procarbazine	+						
Camptothecin	+						
Actinomycin D	−						
Daunorubicin		±					
Cinerubin A		±					
Prednisone†							

*+ = therapeutic synergism, ± = combination marginally better than single agents, − = combination no better than single agents. Tumor systems: L1210 = L1210 leukemia; P388 = P388 leukemia; B16 = B16 melanoma; C3H = C3H mammary carcinoma; ROS = Ridgway osteogenic sarcoma; AKR = spontaneous lymphoma of AKR mice; LL = Lewis lung carcinoma.

† No synergism in P1798 lymphosarcoma.

Investigations of adriamycin in combined modalities with surgery, radiation, and immunotherapy will, undoubtedly, also provide data that will be highly relevant to the clinic.

In summary, it is clear that adriamycin employed individually or in combination with other drugs has therapeutic properties reflecting great potential for clinical usage. It is the function and obligation of preclinical investigators to provide fundamental information pertaining to avoidance of undesirable host toxicity and to improvement of therapeutic response. In order to optimize clinical utilization, emphasis is currently being placed on further elucidation of the mechanism of action and the determination of pharmacologic and biochemical characteristics of adriamycin, employed alone or in combination with other drugs or modalities. The current interest in adriamycin and the knowledge forthcoming from detailed investigations with the drug will surely lead to important further advances in the treatment of clinical neoplasia.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8

Adriamycin-14-octanoate: NSC-149584; adriamycin, 14-octanoate, hydrochloride

5-Azacytidine: NSC-102816; CAS reg. No. 320-67-2; *s*-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis-(2-chloroethyl)-1-nitroso-

Camptothecin: NSC-100880; CAS reg. No. 25387-67-1

CCNU: NSC-79037; CAS reg. No. 13010-47-7; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Cinerubin A: NSC-18334

Cyclocytidine: NSC-145668; CAS reg. No. 10212-25-6; 2*H*-furo[2′,3′:4,5]oxazolo-[3,2-*a*]pyrimidine-2-methanol, 2,3,3a,9a-tetrahydro-3-hydroxy-6-imino-, monohydrochloride

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6

Ellipticine: NSC-71795; CAS reg. No. 519-23-3; 6*H*-pyrido[4,3-*b*]carbazole, 5,11-dimethyl-

Emetine: NSC-33669; CAS reg. No. 316-42-7; emetine, hydrochloride

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

Ftorafur: NSC-148958; CAS reg. No. 17902-23-7; uracil, 5-fluoro-1-(tetrahydro-2-furyl)-

ICRF-159: NSC-129943; CAS reg. No. 21416-87-5; 2,6-piperazinedione, 4,4′-propylenedi-, (±)-

Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-; L-phenylalanine mustard

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

Mitomycin C: NSC-26980; CAS reg. No. 50-07-7

Neocarzinostatin: NSC-157365; CAS reg. No. 4507-13-5

NSC-45388: CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-

PalmO-ara-C: NSC-135962; CAS reg. No. 31088-06-9; cytosine, 1-β-D-arabinofuranosyl-, 5′-palmitate

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

ThioTEPA: NSC-6396; CAS reg. No. 52-24-4; phosphine sulfide, tris(1-aziridinyl)-

Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Review of the Survival and Cell Kinetics Effects of Adriamycin (NSC-123127) on Mammalian Cells[1,2]

S. C. Barranco[3,4,5]

SUMMARY

Adriamycin is extremely toxic to dividing and nondividing Chinese hamster ovary cells in relatively low drug concentrations. The survival response of dividing and nondividing cell populations is biphasic. Dividing cells in mitosis and early S phase are most sensitive to the drug: G_1 and late S-phase cells exhibit the least sensitivity. Drug-induced progression delay is observed in all phases of the cell cycle except mitosis. The length of delay appears to be dose dependent. Both dividing and nondividing cells experience a 1.5-3-fold greater reduction in survival when adriamycin is administered in two small dose fractions (separated by 1-4 hours) than when the same total dose is administered at one time. The fractionated dose technique may represent a means of producing a greater cell kill with lower doses of the drug, while reducing the toxic side effects to the patient.

[Cancer Chemother Rep Part 3, vol 6:147-152, 1975]

Adriamycin is an anthracycline antibiotic isolated from *Streptomyces peucetius* var. *caesius* (1). It is closely related to another antibiotic, daunorubicin,[6] differing only in replacement by a hydroxyl group of a hydrogen atom in the acetyl radical of the aglycone moiety (2). While adriamycin is not active as an antimicrobial agent (3), it has been shown to exhibit antitumor activity with a high therapeutic toxicity ratio (2,4). The drug has been shown to interfere with DNA and RNA synthesis, while protein synthesis appears to be unaffected (5,6). It has also been reported that adriamycin kills cells most effectively in S phase (5).

The purpose of this paper is to review the effects of adriamycin on cell survival, stage sensitivity, and cell progression throughout the cell cycle of Chinese hamster ovary (CHO) cells growing in vitro.

MATERIALS AND METHODS

Preparation of Adriamycin

Adriamycin manufactured for experimental use (Soc. Farmaceutici Italia An.p.Az., Milan, Italy) was used in these experiments. The solutions were always made immediately prior to use by dissolving the drug in sterile 0.9% NaCl solution and diluting into the growth medium. The pH of the treatment medium was adjusted to 7.2-7.4.

Cell Culture Techniques

CHO cells were grown exponentially in monolayer cultures as described by Humphrey et al (7). Under these growing conditions the average cell cycle time was determined to be 12.5 hours. The pre-DNA synthesis period (G_1) was 2.5 hours in length, DNA synthesis (S) 8 hours, post-DNA synthesis (G_2) 1.5 hours, and mitosis (M) 0.5 hours. Experiments involving asynchronous populations were performed on cells in logarithmic phase growth. In cell progression experiments involving the assay of radioisotope-labeled cells, liquid emulsion (Ilford K 5) autoradiography techniques were used. In all experiments involving single-cell survival, viability was measured by the ability of a cell to form a colony of 50 or more cells. A known number of single cells were plated into Petri dishes containing growth media and incubated at 37°C for 6-8 days. Colonies were stained with crystal violet and counted. Plating efficiencies in all cases were greater than 80%.

The techniques for obtaining synchronized or nondividing cells and for fractionation of drug doses have been reported elsewhere (8-10).

RESULTS AND DISCUSSION

Survival of Asynchronous CHO Cells

The survival of CHO cells following a 1-hour treatment with varying doses of adriamycin is shown in figure 1. The survival response to this drug

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[2]Adriamycin: CAS reg. No. 23214-92-8.

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[6]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

is biphasic (n = 1, D_o = 0.2 μg/ml for 1 hour in about 99% of the population and D_o = 9.4 μg/ml for 1 hour in the more resistant group of cells). Survival is reduced to less than 0.2% with a dose of 2 μg/ml of adriamycin. No shoulder is observed in the response curve in the dose range from 0 to 2 μg/ml of adriamycin. At doses greater than 5 μg/ml, the survival response of the remaining resistant 0.1% of the population assumes a shallow shape through a dose of 25 μg/ml. Therefore, the doses greater than 5 μg/ml are wasted in the sense that they may increase the chances of toxic side effects to the patient without effecting a greater cell kill.

A comparison of the high killing efficiency of adriamycin on dividing and nondividing cells in vitro is shown in figure 2; the results suggest that this agent may be an effective drug for use on slowly growing solid tumors containing large fractions of nondividing cells. We are aware of the limitations inherent in comparing plateau phase cells in vitro with G_0 cells in vivo. However, these results do suggest the possibility that considerable killing in the G_0 compartment in vivo may be observed. A better understanding of the effects of anticancer drugs on these viable but nondividing cell populations has potential clinical significance. Since the plateau or G_0 cells generally are not killed by the cell cycle-specific drugs, these cells probably contribute to the repopulation or regrowth of the tumor. Therefore, it is extremely important to identify and characterize drugs that will specifically influence the survival, repair, and repopulation kinetics of G_0 and plateau phase cells. Adriamycin appears to be such a drug.

Survival Response of Synchronized Cells

In order to determine the relative sensitivities to adriamycin during each stage of the cell cycle, survival data were obtained on synchronous populations of cells. In these experiments, the location of the cells within the cell cycle at the time of treatment was determined using labeling (^{3}H-thymidine) and mitotic index data. The sensitivity of mitotic cells is depicted in figure 3. Drug concentrations of 0.1 μg/ml (asynchronous survival = 67%) and 0.5 μg/ml (asynchronous survival = 8%) were used. Although approximately one half of the cells were killed regardless of the dose or position in the cell cycle, some stages were more sensitive than others. Mitotic cells treated with adriamycin showed a dose-dependent reduction in survival: 30% survival when treated with 0.1 μg/ml and 2.3% survival following treatment with 0.5 μg/ml. In comparison to M cells, G_1-phase cells were less sensitive to both treatment doses.

Other populations of synchronized cells were treated for 1 hour at hourly intervals throughout S and G_2 phases of the cell cycle. The dose-dependent survival responses of cells treated in S and G_2 phase are shown in figure 4. Cells treated in early S phase show the greatest sensitivity to the drug at both doses used. Treatment with 0.01 μg/ml adriamycin resulted in 29% survival while the 0.5-μg/ml treat-

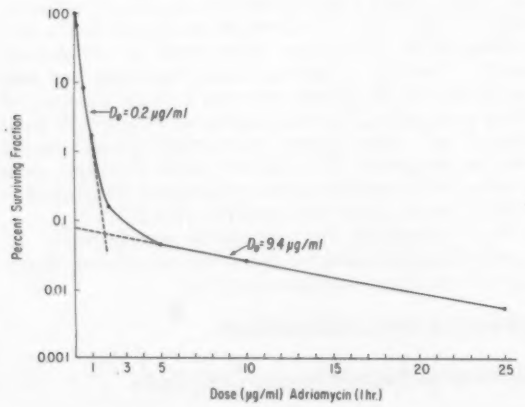

FIGURE 1.—Effect of adriamycin on survival in asynchronously growing CHO cells.

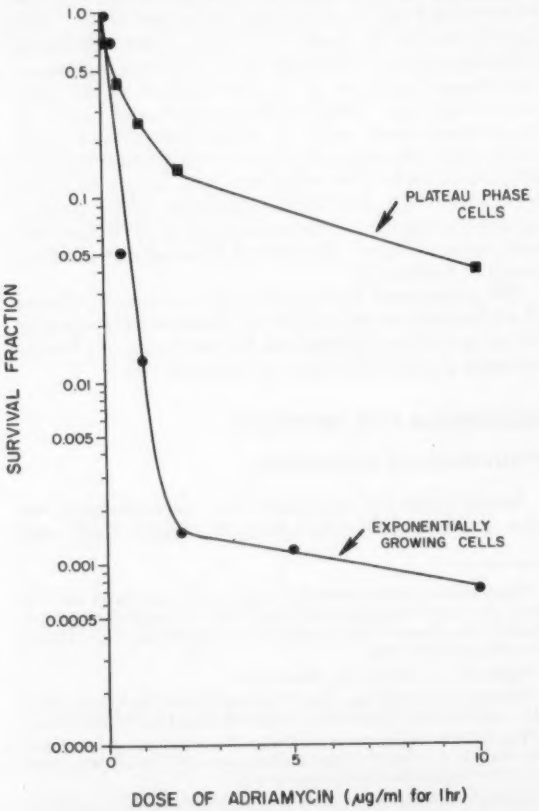

FIGURE 2.—Survival responses to adriamycin of dividing and nondividing CHO cells.

ment resulted in 2.4% survival during the second hour of S phase. The survival response of early S-phase cells resembles the sensitivity of mitotic cells. In contrast, late S-phase cells are less sensitive to both doses used.

Since cells are killed in all phases of the cell cycle and in the nondividing state, adriamycin cannot be classified solely as a cell cycle-active or a phase-specific drug. Instead, the data suggest adriamycin to be a drug of choice for slowly growing tumors with low growth fractions.

Progression of Synchronized Cells Treated With Adriamycin

Adriamycin caused progression delay in all phases of the cell cycle except mitosis. The length of progression delay is dose dependent, as seen in figures 5-8. Cells treated continuously in G_1 with 0.1 μg/ml were delayed 1 hour but then proceeded normally into S phase (fig 5). G_1 cells treated continuously with 0.5 μg/ml were delayed for 3 hours and then progressed erratically and at a much slower rate into S phase. The progression delay of G_1 cells may be due to dose-dependent interference of normal RNA activity or synthesis in this phase of the cell cycle. The drug has been shown to rapidly inhibit RNA synthesis (5,11). Furthermore, several investigators (12-14) have presented evidence that the inhibition of RNA synthesis in G_1 phase delays the initiation of DNA synthesis. Why some of these G_1 cells were able to overcome the progression block and move into S phase is unclear; however, it may be the result of drug inactivation or decay.

CHO cells treated with 0.5 μg/ml adriamycin in early S phase (fig 6) exhibited complete blockage in S phase regardless of whether the treatment lasted for 1 hour or was given continuously. However, cells treated continuously in early S phase with 0.01 μg/ml were delayed in S phase for only 2 hours and then progressed into G_2 (fig 7). Our data at the cellular level do not indicate the cause of the S-phase progression delay; however, Wang et al (11) have shown that adriamycin inhibits DNA polymerase activity, probably by binding to the template DNA. Since they also showed that the inhibition of DNA polymerase was dose dependent, it is possible that adriamycin may cause the cells to be irreversibly blocked

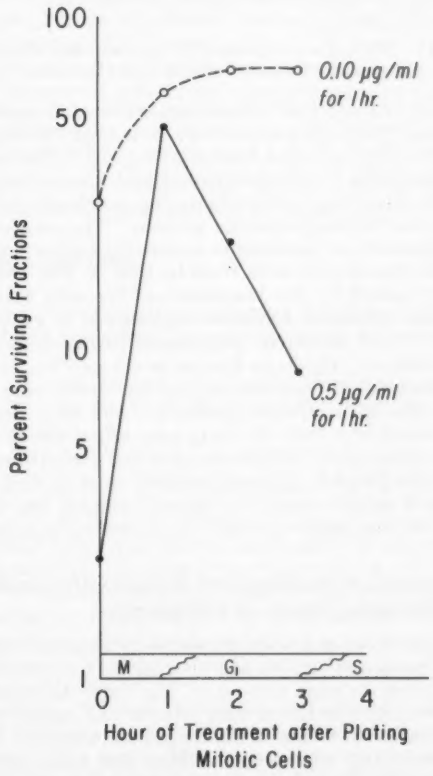

FIGURE 3.—Effect of adriamycin on survival of synchronized CHO cells treated in mitosis or G_1 phase.

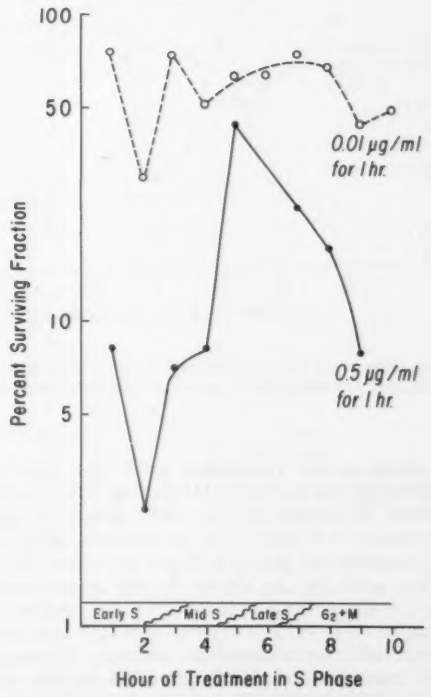

FIGURE 4.—Adriamycin effects on survival of synchronized CHO cells treated in S or G_2 phase.

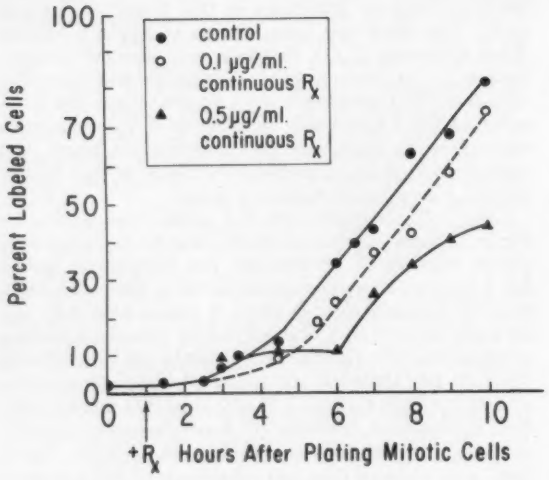

FIGURE 5.—Adriamycin effects on cell progression from G_1 into S phase.

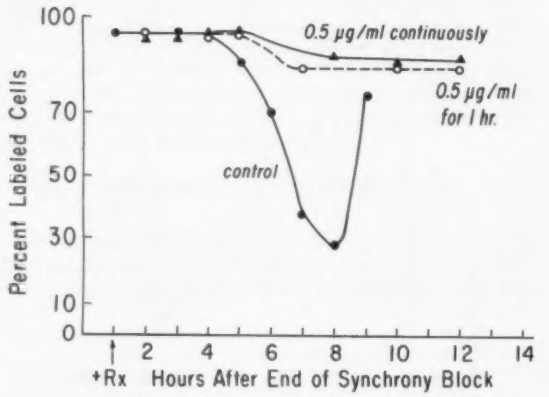

FIGURE 6.—Effects on cell progression from S into G_2 phase after adriamycin treatment with 0.5 μg/ml for 1 hr or continuously.

in S phase after treatment with the high dose (0.5 μg/ml) by irreversibly inhibiting DNA polymerase, while treatment with a lower dose (0.01 μg/ml) would result in a short, but reversible, delay in S-phase progression due to a lesser effect on DNA polymerase activity. As shown in the lower panel of figure 7, S-phase cells treated with the smaller dose (0.01 μg/ml) also exhibited some difficulty in progression through the subsequent mitosis. The control mitotic index peaked at 8 hours (20% mitotic index) and fell quickly to 3%. The mitotic index of the treated cells, however, reached a maximum at 9 hours and decreased at a much slower rate.

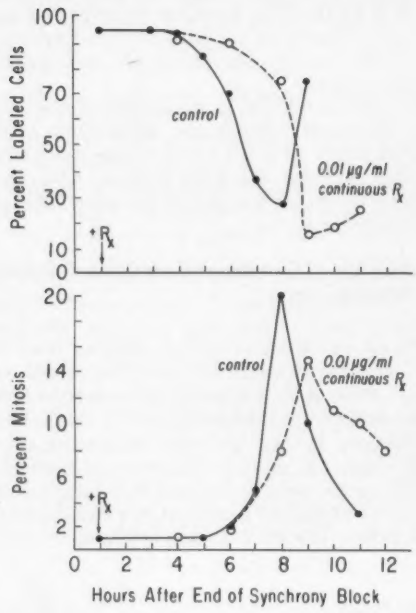

FIGURE 7.—Effect of a continuous 0.01- μg/ml adriamycin treatment on progression from S into G_2 and M phases.

Cells in G_2 at the time of treatment with adriamycin are immediately prevented from progressing into mitosis (fig 8). It has been shown (15-17) that interference with the translational and transcriptional events occurring in G_2 affects the synthesis or function of a "division-specific protein." The presence of this protein (or proteins) is necessary for the successful progression of cells from G_2 into M. The onset of delay caused by the treatment of G_2 cells with the protein synthesis inhibitor puromycin or cycloheximide (16,17) produced a G_2 block 10-25 minutes before mitosis. Although Kim et al (5) and Wang et al (11) showed that adriamycin produced only very minimal effects on protein synthesis, our data suggest the possibility that the drug may affect the synthesis or structural configuration of the proteins necessary for division and progression, since adriamycin-induced delay occurs at approximately the same point in G_2 phase as that produced by puromycin (16,17).

Response of Dividing and Nondividing Cells to Fractionated Doses of Adriamycin

Adriamycin has been shown to be cleared rapidly from the blood (18,19), but is retained for extremely long times in most tissues of the body. As a result, severe dose-dependent side effects can result from treatment with this drug, including alopecia, nausea, vomiting, myelosuppression, and heart irregularities (20). It is important, therefore, to find ways of reducing the level of toxicity of adriamycin without reducing the cell-killing effects. The data pre-

sented in the lower panel of figure 9 suggest that this may be accomplished through dose-fractionation techniques. It can be seen that the dividing cells are more sensitive (threefold reduction in survival) to two dose fractions of adriamycin (5 μg/ml for 30 minutes) than to a single dose of 5 μg/ml for 60 minutes. In fact, the survival fraction obtained after two dose fractions of 5 μg/ml for 30 minutes (fig 9) is equivalent to that resulting from a treatment with a 25-μg/ml dose for 1 hour (fig 1). Thus, the same reduction in survival was obtained (fractionated treatment) using a 2.5 times lower dose of the drug. Since the side effects appear to be dose dependent, it may be possible to reduce them while achieving the same amount of cell kill through the use of fractionated dose treatments. Similar responses are observed in nondividing cells (lower panel of fig 10).

It should be noted from the upper panels of figures 9 and 10 that the response to fractionated dose treatments is also dose dependent. By using low doses of adriamycin, the responses to dose fractionation may fluctuate or, as in the case of the nondividing cells (upper panel of fig 10), actually suggest recovery of drug-induced damage (increased survival). It should also be noted that the time between the two doses is only 1-4 hours. This is a departure from the usual protocol schemes where the drug doses are administered 12-72 hours apart.

A great deal more study is required to explain the dose-dependent differences in response to fractionated doses of adriamycin and to determine the relevance of the time interval between the dose fraction; however, it is important to continue such studies since they may result in methods for reducing toxicity while retaining a high cell-killing potential.

CONCLUSIONS

The following conclusions may be made from the effects of adriamycin on cell survival and division delay:

1. Adriamycin causes dose-dependent cell progression kinetics delays in all stages of the cell cycle except mitosis.

2. Adriamycin cannot be considered a phase-specific or cell cycle-specific drug since it kills cells in all phases of the cell cycle and in the nondividing state. However, dividing cells are more sensitive than nondividing cells, and mitosis and early S phase are more sensitive than the other stages of the cell cycle.

3. At doses greater than 5 μg/ml, there is a drastic reduction in the killing ability of adriamycin. Higher doses may be considered, therefore, to be wasted and harmful since they would increase the severity of the side effects.

4. Small fractionated doses of adriamycin are more effective at reducing cell survival than are larger single doses. This technique may serve as a means for achieving greater cell kill with lower doses of adriamycin, thus reducing the total dose of the drug and, therefore, the side effects.

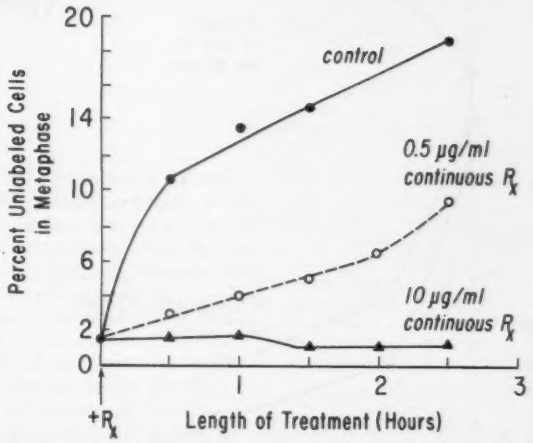

FIGURE 8.—Adriamycin effects on progression of cells from G_2 into mitosis after treatment of asynchronous cells in G_2 phase.

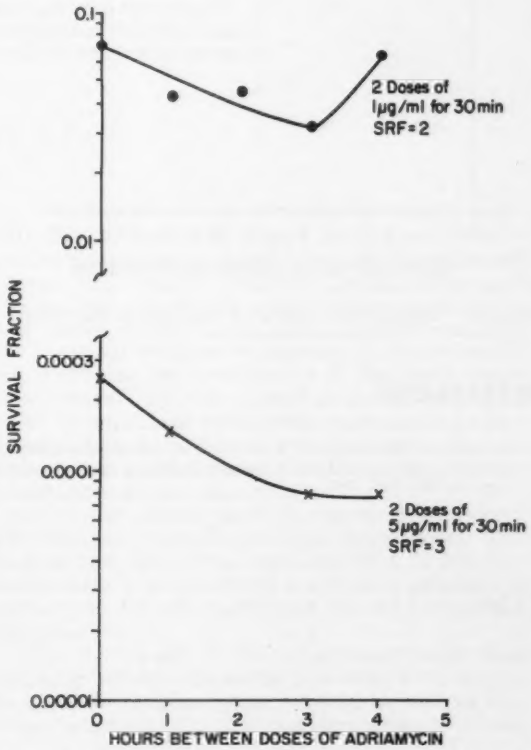

FIGURE 9.—Responses of dividing CHO cells to fractionated doses of adriamycin.

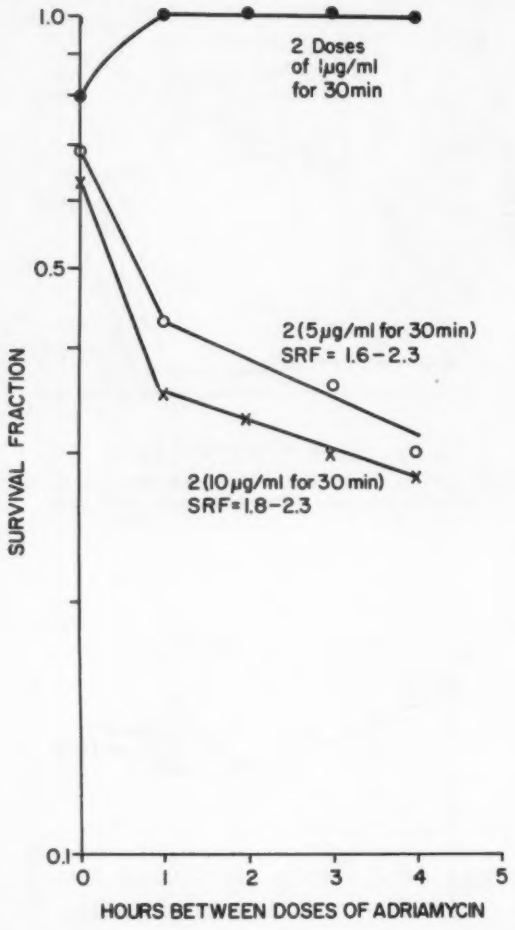

FIGURE 10.—Responses of nondividing CHO cells to fractionated doses of adriamycin.

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Adriamycin (NSC-123127) Pharmacology[1]

Nicholas R. Bachur[2,3]

SUMMARY

Adriamycin is a drug of multipotential molecular character. Embodied in a single molecule are centers for lipophilic and hydrophilic interaction, acidic and basic binding, and several sites for biotransformation. Therefore, the variety of interactions of this drug with nucleic acids, lipids, proteins, and supportive matrices in biologic systems is to be expected. Adriamycin's complex pharmacokinetics and pharmacodynamics reflect these potentials. The drug enters cells rapidly through an active process and concentrates in the nuclei. Adriamycin and its metabolites are measurable in all tissues, with the highest concentrations in the lung, kidney, spleen, small intestine, and liver, and with the lowest concentrations in the brain. Binding to cellular components causes a relatively long plasma half-life, although excretion via the bile and urine is active. Biliary excretion of this agent is important to its removal from the body, and any compromise to this system can cause greater drug toxicity. In the intestine the drug and its polar metabolites are degraded to the aglycone level. Metabolism occurs rapidly and primarily intracellularly. Enzymatic keto reduction to the pharmacologically active product adriamycinol is the major step in biotransformation and occurs in the cellular cytoplasm of most cells. At the microsomal level, the drug is deglycosidated, O-demethylated, and conjugated with sulfate or β-glucuronic acid. Metabolites reflecting these reactions and several unidentified ones are detected in animal and human bile and urine as well as in animal tissues. The contributions of drug metabolism, tissue binding, membrane transport, physiologic disposition, immunologic suppression, and other factors cannot be neglected in understanding adriamycin's important pharmacologic activity against cancer cells or its serious toxic side effects.

[Cancer Chemother Rep Part 3, vol 6:153-158, 1975]

This review focuses on the disposition and metabolism of adriamycin in mammals and in in vitro systems. Our work on the human pharmacology of this drug will be discussed in other papers presented at this symposium by Dr. Robert S. Benjamin.

Judging from the conflicting observations and reports on the disposition and fate of adriamycin, confusion abounds. This is understandable considering the chemical nature of the substance. If we examine adriamycin's molecular structure (fig 1) we can appreciate its multipotential character and the complexity of its pharmacologic effects and biochemical activities. The molecule consists of a napthacenequinone nucleus linked through a glycosidic bond at ring atom 7 to the amino sugar, daunosamine. Two major centers of physicochemical activity dominate the molecule. The planar anthracycline resonating ring system is lipophilic, but the saturated end of the ring system contains abundant hydroxyl groups adjacent to the amino sugar producing a hydrophilic center. If the amino sugar is removed, the resulting adriamycin aglycone is water insoluble, indicating the contribution of the amino sugar for compatability with aqueous systems. In addition to its lipophilic and hydrophilic characteristics, the molecule is amphoteric and contains both acidic and basic functions in the ring phenolic groups and the sugar amino group respectively.

As a result of both polarity and charge, adriamycin binds to numerous biologic components. The binding to nucleic acids, especially DNA, is well documented (1). Less recognized are binding affinities of adriamycin for cell membranes (2) and for plasma proteins.[5]

Both in vivo and in vitro experiments show that splitting the molecule at the glycosidic bond results in a loss in pharmacologic activity as well as biochemical activity (3).[6] This is understandable in view

[1]Adriamycin: CAS reg. No. 23214-92-8.

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[3]Credits are due to many of my colleagues and co-workers for their contributions to this program: Dr. Delano Meriwether, Dr. David Huffman, Dr. Shigeru Takanashi, Dr. Merrill Egorin, Dr. Emanuela Arena, Dr. Peter Wiernik, Mr. Charles Riggs, Mr. Robert Hildebrand, and Mr. Malcolm Gee.

[4]*Reprint requests to:* Dr. Nicholas R. Bachur, Biochemistry Section, Laboratory of Pharmacology, BCRC, DCT, NCI, 3100 Wyman Park Dr, Baltimore, Md 21211.

[5]Axelrod M, Kaber M, and Bachur NR. Unpublished observations.

[6]Bachur NR. Unpublished observations.

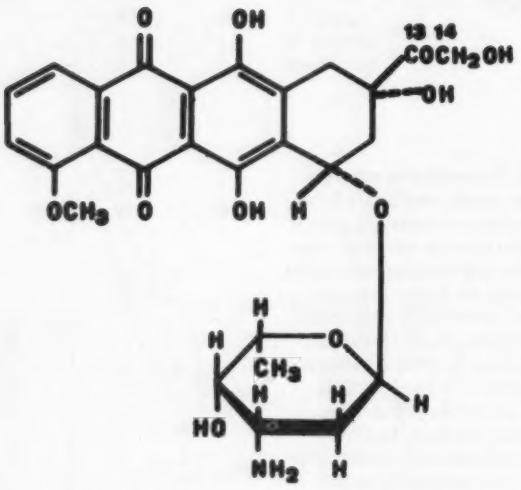

FIGURE 1.—Adriamycin free base structure.

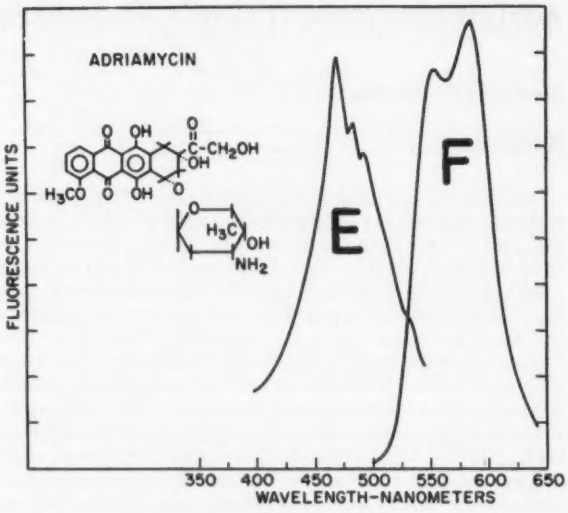

FIGURE 2.—Fluorescence spectra of adriamycin; E = excitation spectrum; F = fluorescence spectrum.

of the proposed mechanism of action of the analog daunorubicin[7] as a DNA intercalating agent provided with stabilization by the amino sugar (4). Splitting the drug also alters solubility, cellular uptake, and binding properties. Because of the lability of the glycosidic bond to enzymatic and acid-catalyzed cleavage, the drug is essentially inactive when administered orally (5). The intravenous (iv) route of administration is used although extravasation into tissues causes tissue necrosis.

The molecular structure also suggests that several sites are available for biotransformation. We will discuss several of these reactions and the resultant complexity of adriamycin disposition.

Several investigators have studied the pharmacokinetics of adriamycin using different assay techniques, including the use of radiolabeled adriamycin (6,7), bioassay of bacterial inhibition (8), and the native fluorescence of the compound (5,9-15). These studies have resulted in divergent findings. In our studies on animal and human pharmacokinetics and pharmacodynamics, we assayed the drug by its native fluorescence which is both sensitive and specific (fig 2). Recently we compared our fluorescence methodology with a newly developed radioimmunoassay for adriamycin (16) and we found excellent correlation.

In our earliest studies on adriamycin pharmacokinetics in man we found rapid and extensive metabolism of the drug resulting in complex pharmacokinetics (9). When the pharmacologic disposition of adriamycin is compared among several species, important differences are noticeable. Since some interspecies differences are seen in metabolism, disposition, and toxicity, we have used the rabbit as a model for pharmacokinetic studies because of the dose-dependent cardiotoxicity which this species develops that emulates the human condition (17,18).

When injected iv into rabbits, adriamycin rapidly leaves the extracellular space and moves into cells (fig 3), as indicated by the large volume of distribution. The long plasma half-life suggests tissue retention and slow excretion. Shortly after administration, a substantial part of the drug in blood is the reduced metabolite adriamycinol. Other plasma metabolites are seen but have not been identified in the rabbit.

When tissues are excised after drug administration and examined by cytofluorescence microscopy, the drug is found almost exclusively in the nuclear structures (19). No redistribution to other cellular organelles is seen. Nuclear localization has also been shown by radioactive tracer labeling and cytofluorescence in HeLa cells in tissue culture (20).

As determined by the in vivo pharmacokinetics and by in vitro transport studies, adriamycin crosses cell membranes readily and is imbibed by an active process against a gradient (2). The uptake process is a temperature-sensitive function similar to the uptake of daunorubicin (21). In addition, the intracellular concentration of the drug often exceeds the plasma concentration by several fold (15).[8]

Adriamycin attains its highest concentration in the lung, kidney, spleen, small intestine, and liver (table 1), and little if any drug is measured in the brain. This impermeability of the blood-brain barrier due to the charged nature of the compound is verified by lack of activity of adriamycin in brain tumors.

[7]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

[8]Harris PA, and Chan KK. Personal communication.

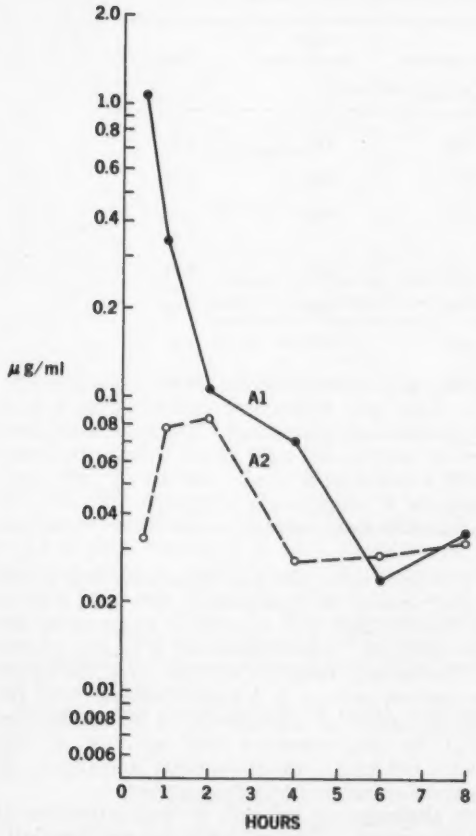

FIGURE 3.—Mean plasma adriamycin (A1) and adriamycinol (A2) concentrations in 4 rabbits given 5 mg/kg iv (*from* ref 15).

Adriamycin metabolites are seen in all tissues. In fact, adriamycinol, while a significant component in all tissues, exceeds the parent drug concentration in the brain and liver. Similar patterns of adriamycin distribution and metabolism are seen in hamsters (14) and rats,[9] although the adriamycinol conversion is less striking in these species. Several other metabolites were identified and measured in the tissues. Aglycones were found chiefly in the liver and kidney, and polar metabolites were significant in the kidney, spleen, and small intestine.

Adriamycin is excreted primarily via the biliary route in all animals examined, ie, rabbit (5,15), dog (13), rat,[9] and man (22), with less excretion in the urine. Several metabolites are seen in rabbit bile, adriamycinol being the most outstanding (fig 4). Polar metabolites and aglycones are also found. Although the urine contains small amounts of the drug, parent drug predominates and adriamycinol is

[9]Cradock JC, Egorin MJ, and Bachur NR. Unpublished observations.

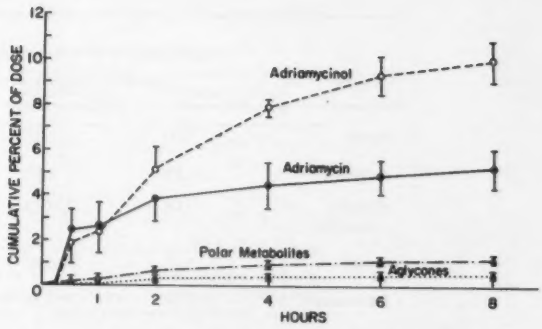

FIGURE 4.—Biliary excretion of adriamycin and its metabolites in rabbits given 5 mg/kg iv (*from* ref 15).

the major metabolite (fig 5). In human urine, adriamycinol, conjugated metabolites, and aglycones are substantial metabolic excretory products and reflect the active metabolism of this agent (23). This dependence on biliary excretion instigated the investigation of the relationship of hepatic dysfunction and adriamycin toxicity in man (24).

After adriamycin and its metabolites enter the intestinal tract via the bile, further biotransformation occurs. As clearly shown for daunorubicin and its metabolites (25) and for adriamycin and its polar metabolites,[9] degradation occurs in the small and large intestine of the rat. As the polar molecules proceed down the intestinal tract, they are metabolically trimmed to yield predominantly aglycones. As a result, aglycones are the major excretory products of the anthracyclines in feces.

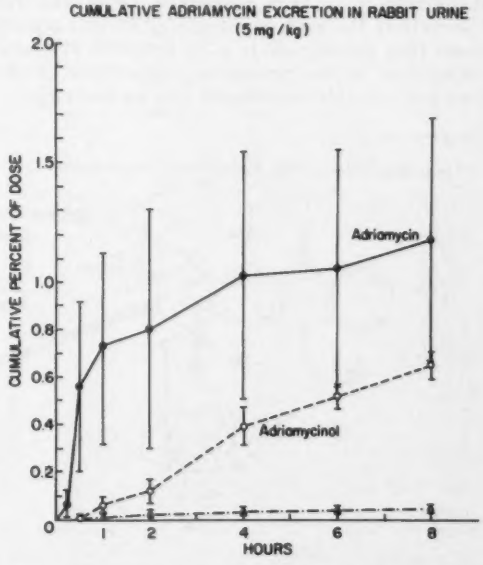

FIGURE 5.—Urinary excretion of adriamycin and its metabolites in rabbits given 5 mg/kg iv (*from* ref 15).

TABLE 1.—8-Hr adriamycin tissue levels in rabbits after doses of 5 mg/kg

Tissue	Aglycones	Adriamycin	Adriamycinol	Polar metabolites	Total
	(μg of adriamycin equivalents/g of tissue wet weight)				
Brain	ND*	ND	0.08	0.07	0.15
Adipose	0.03	0.37	0.04	ND	0.44
Skeletal muscle	0.04	1.12	0.18	0.01	1.34
Heart	0.01	2.63	0.25	0.01	2.91
Pancreas	0.05	2.40	0.92	0.02	3.40
Liver	0.85	1.95	1.97	0.07	4.84
Small intestine	0.13	4.63	1.79	0.44	6.99
Lung	0.03	6.13	1.03	0.04	7.22
Spleen	0.12	14.70	4.30	0.26	19.40
Kidney	0.94	16.80	13.80	0.45	31.90

*ND = not detectable.

Most of the metabolism of adriamycin occurs intracellularly, although some degradation of the molecule occurs in plasma, urine, and bile. Intracellular cytoplasmic aldo-keto reductase is the major catalytic factor in adriamycin metabolism and converts the drug to adriamycinol (fig 6) (15).

In a study of the comparative aldo-keto reductase activities in animal tissues, the kidney contains the highest activity levels, although the enzyme is found in all the tissues examined. The study also showed that daunorubicin is a preferred substrate over adriamycin.[10] However, our studies in animals and man indicate that the enzyme is quite active in vivo since adriamycinol is the prominent metabolite. Tumor tissues also contain significant enzyme activities.

An aldo-keto reductase has been isolated from rat liver and purified to homogeneity (26). This enzyme is specific for NADPH as a cofactor, has a molecular weight of 39,500, and consists of a single peptide unit. The normal function of this constitutive enzyme is as yet unclear. It is clear, however, that the pharmacokinetics of adriamycin in all species are affected by the presence and activity of the aldo-keto reductase which converts adriamycin to its more polar metabolite, adriamycinol.

Since adriamycinol has only a small alteration of the D ring which apparently does not substantially affect interaction with DNA, the metabolite retains its ability to inhibit DNA and RNA metabolism in cultured L1210 cells (table 2). The total effects of the aldo-keto reductase on adriamycin pharmacokinetics and pharmacodynamics have not yet been elucidated and remain a major challenge.

[10]Arena E, and Bachur NR. Unpublished observations.

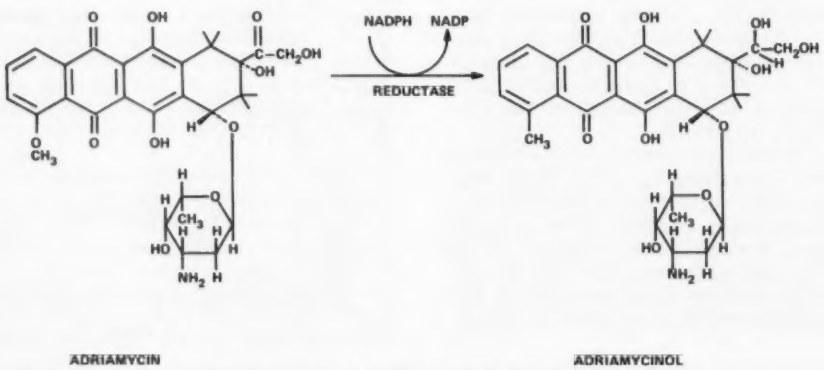

FIGURE 6.—Adriamycin reduction by aldo-keto reductase.

TABLE 2.—Effects of adriamycin and adriamycinol on L1210 cell nucleic acid metabolism*

	Concentration (μM)	Percent inhibition of—	
		^{14}C-uridine-incorporation	^{3}H-thymidine-incorporation
Adriamycin	1.0	22	29
	5.0	57	52
Adriamycinol	1.0	10	24
	5.0	23	32

*Cultured L1210 cells (10^6) were incubated at 37°C in 1 ml of media containing the drug for 1 hour. Radioactive uridine or thymidine was added and the incubation was continued for another hour. The incorporation of radiolabel was determined as previously reported (2).

Several particulate enzymes metabolize adriamycin and adriamycinol. Two major ones are a reductive glycosidase and a hydrolytic glycosidase which remove the amino sugar from the anthracycline nucleus (fig 7) (27-29). Both glycosidases require NADPH. The reductive glycosidase, a microsomal enzyme, is inhibited by oxygen, and although it is found in most tissues it is very active in the liver (27). This enzyme prefers daunorubicin to adriamycin as a substrate although adriamycin is split substantially as evidenced by the aglycone products seen in vivo. The glycosidases yield products which have little activity against nucleic acid metabolism and are not cytotoxic.

Evidence for participation of other enzymes in adriamycin biotransformation can be ascertained from other metabolites detected in bile and urine (fig 7) (23). Apparently the water-insoluble aglycone metabolites are resolubilized by conjugation steps. The aglycone products of the glycosidases are preferentially demethylated at the ring 4 position to yield demethyl deoxyadriamycinol aglycone (VI) (fig 7). This aglycone is conjugated to a sulfate or glucuronic acid. Both rabbits and man produce substantial quantities of these conjugates in urine and bile.

Although the aglycone metabolites have been tested for activity in vivo in cell culture and in enzymatic systems, they retain little pharmacologic or biochemical activity. They may have activity when liberated intracellularly. Once these compounds are

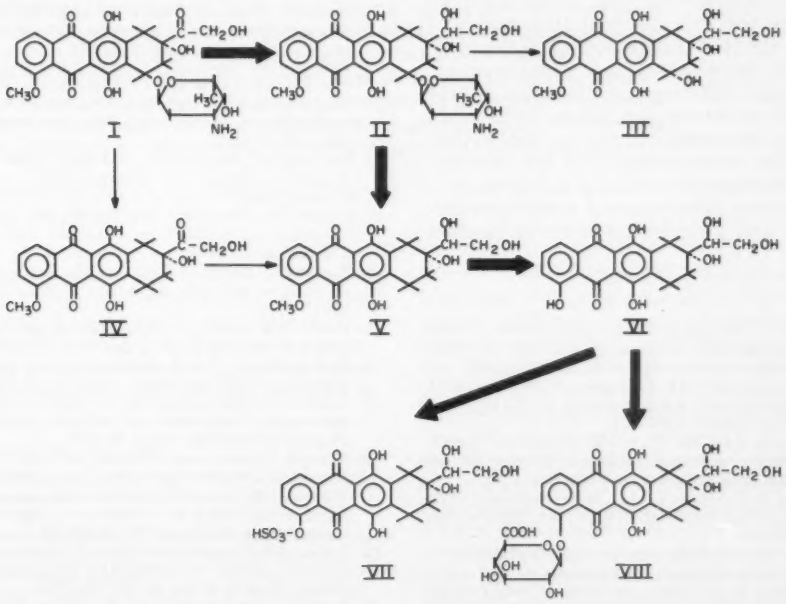

FIGURE 7.—Adriamycin metabolic pathway in man (*from* ref 23).

conjugated to sulfate or glucuronic acid, it is unclear whether they have any pharmaçologic or toxicologic activity as the conjugate.

Although this biotransformation scheme is already complex, it probably is only partially complete since we have detected several other unidentified metabolites in rabbit and human urine.

The drug-drug interactions of adriamycin have only been scantily studied (30,32). Arena et al (30) studied the effects of strophanthin G on cardiac function in adriamycin-treated animals and found significant effects. It has also been shown, however, that strophanthin G pretreatment does not inhibit adriamycin accumulation in the heart (31). Since adriamycin is very dependent on intracellular metabolic activities, it is important to determine the effects of activators and inhibitors on the pharmacologic action of this drug.

Drs. Di Marco and Chandra have compared the structures of adriamycin and its analogs to their activities against viral replication and enzymatic synthesis of DNA and RNA. Under these in vitro conditions, adriamycin and daunorubicin are quite similar in their activities. In studies with cultured cells, daunorubicin has greater activity than adriamycin as we originally described (2). This perplexing result is consistently seen in all experiments where intact cultured cells are compared and is caused by a greater rate of uptake and accumulation of daunorubicin in the cells (2). If we compare these structure-activity relationships in a broader scope, however (eg, the intact animal), adriamycin always has a higher therapeutic index and is more potent on a molar basis than is daunorubicin. It is apparent that differences in the side chain at ring carbon 9 profoundly affect the therapeutic index and the spectrum of activity of these agents. It is equally apparent that this small difference profoundly affects metabolism, membrane transport, physiologic disposition, immunologic suppression, and probably other factors as well. The comprehension of the pharmacokinetics and pharmacodynamics of adriamycin is apparently of extreme importance in order to understand its unique and complicated activity against cancer cells.

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Pathologic Effects of Adriamycin (NSC-123127) in Experimental Systems[1]

David M. Young[2,3,4]

SUMMARY

Although this review considers some of the more infrequently occurring or unusual histocytopathic effects of adriamycin, it is imperative to mention that myelosuppression, stomatitis, and digestive disturbances are the major clinical toxic effects of adriamycin in patients. The reported incidence of adriamycin-induced cardiotoxicity varies from 1% to 30% depending upon the total cumulative dose administered. In vitro and in vivo studies of the cytotoxic effects of adriamycin have contributed to a better understanding of its mechanism of action on normal and neoplastic cells. Numerous pharmacologic and toxicologic investigations have been performed using various animal species. Since the first report in the United States of the cardiotoxic effects of daunorubicin in 1969, investigators have undertaken a search for an animal model with a cardiotoxic syndrome similar to that reported in man. An animal model could be used to test analogs of adriamycin for cardiotoxic potential, to test antidotal procedures, and to study the pathogenesis of the drug-induced cardiomyopathy. Current evidence suggests that the rabbit, monkey, rat, mouse, and hamster develop characteristic, delayed, dose-related, myocardial changes in response to adriamycin administration. Only the rabbit and monkey have been reported to exhibit a congestive heart failure syndrome associated with the cardiomyopathy. Characteristic myocardial lesions include vacuolization, edema, myocytolysis, and fibrosis. Ultrastructural changes have been described in nuclear, myofibrillar, mitochondrial, and sarcotubular organelles. The end result is a complete destruction and lysis of individual cardiac myocytes with replacement fibrosis. The pathogenetic mechanism(s) of the adriamycin-induced cardiotoxicity appears to be multifactorial, involving biochemical and morphologic lesions of the myocardium. Experimental animals appear to be sensitive to the nephrotoxic effects of adriamycin, whereas altered renal function or morphology in patients is rare. An additional adriamycin-induced toxic effect that has not been reported in man is impairment of chondro-osteogenesis. Animal studies have revealed an infrequent carcinogenic effect which has not been reported in man. Hyperpigmentation, allergic reactions, and anaphylaxis have been reported to occur in association with adriamycin treatment. Further studies are required to elucidate the mechanisms involved in the development of these interesting, albeit infrequent, histocytopathologic and biochemical adverse effects of adriamycin.

[Cancer Chemother Rep Part 3, vol 6:159-175, 1975]

During the past 3 years the clinical interest in adriamycin has increased tremendously. Its broad spectrum of oncolytic activity in a wide range of tumor types is well known (1,2). Studies involving adriamycin have been the topic of numerous scientific meetings and seminars in the United States and Europe. The result of this concentration of interest in a single antineoplastic agent has been the production of numerous reports of experimental, preclinical, and clinical data and the approval by the Food and Drug Administration for commercial distribution of the drug in the US. Although considerable interest has been directed at therapeutic effectiveness, adriamycin does possess a number of adverse side effects. A complete knowledge of the predictability, manageability, and reversibility of these adverse effects is of paramount importance in clinical studies. Few reports have directed their attention to a comprehensive review of the toxic effects of adriamycin in experimental animals and patients with an emphasis on pathogenetic mechanisms. The objectives of this report are to review the more infre-

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quently occurring clinicopathologic and histocytopathologic effects of adriamycin and to present recent findings of adriamycin studies in our laboratory.

IN VITRO STUDIES

Numerous biochemical studies have resulted in the thesis that adriamycin acts at the cellular level by intercalating between base pairs of DNA and inhibiting RNA in a manner similar to daunorubicin.[5] Investigations of the effects of adriamycin on cells in culture have been the topic of a number of studies (3-7). Utilizing HeLa cell cultures, Silvestrini et al (3) concluded that adriamycin rapidly penetrates cells and binds to nuclear structures with a marked localization near perinuclear chromatin.

Other culture studies have been designed to determine if adriamycin possesses cell-cycle-specific lethality. The results of studies by Wheatley (4) indicate that adriamycin prevents cells from progressing through the G_2 interval into mitosis, thus providing a mode of action which is considered "radiomimetic" in nature. Kitaura et al (5) reported similar findings that adriamycin prevents HeLa cells from entering mitosis. They also observed that adriamycin inhibited RNA synthesis more markedly than DNA synthesis in logarithmically growing cultures. Kim et al (7) found adriamycin to be less effective than daunorubicin on a molar basis in killing HeLa cells in culture. Studies of Chinese hamster ovary monolayer cultures revealed that cells in mitotic and S phases are highly susceptible to the lethal effects of adriamycin, although adriamycin could not be considered a true cell-cycle-phase-specific drug (8). These findings were corroborated by Drewinko et al (9) who concluded from their studies that adriamycin used clinically could hardly be considered a phase-sensitive drug. Wang et al (10) utilized adriamycin-incubated L1210 leukemia cell suspensions to show a marked inhibition of DNA polymerase activity and a lack of incorporation of precursors into DNA and RNA. They concluded that both daunorubicin and adriamycin inhibit RNA and DNA synthesis. Tatsumi et al (11) also reported decreased DNA polymerase activity in L1210 leukemia cells incubated with adriamycin. Daunorubicin appeared to be superior to adriamycin in inhibiting DNA and RNA synthesis and in total uptake of drug by leukemic cells.

A number of investigators have studied the effects of adriamycin on human blood and bone marrow cells (10,12-14). Boll (12) reported that adriamycin produced decreased regeneration of normal granulocytes, increased appearance of binucleated granuloblasts, and disturbance of the enucleation of mature erythroblasts. Massimo et al (14) also observed a constant inhibition of blastogenesis and the appearance of giant cells in lymphocyte cultures treated with adriamycin. Recent comparative studies by Haskell et al (15) using adriamycin in normal and neoplastic human cells in culture revealed inhibition of sarcoma cells, melanoma cells, normal muscle cells, and normal skin fibroblasts in decreasing order of sensitivity. Girardi heart cells were uniquely sensitive to adriamycin, although the reason for this susceptibility was not readily apparent.

Few studies of subcellular morphologic alterations induced by adriamycin in cell cultures have been reported. Vig (13), Massimo et al (14), and Stacher et al (16) have investigated the ability of adriamycin to induce chromosomal aberrations in lymphocytes. Chromosomal fragmentations, rearrangements, chromatid exchanges, polyradial figures, rupture of centromeres, and alteration of chromatid morphology were observed in adriamycin-treated lymphocyte cultures. A detailed study by Stacher et al (16) revealed that chromosomal aberrations were dose dependent between 0.01 and 0.3 mg of adriamycin/ml of culture media. Doses in excess of 0.3 mg/ml resulted in no appearance of mitoses in lymphocyte cultures. They concluded that adriamycin acts during the S phase of the cell cycle and produces up to 98% chromosomal aberrations in cultures. These findings were interpreted as evidence for the mutagenic action of adriamycin.

IN VIVO STUDIES

The morphologic alterations of chromatin induced by adriamycin correlate well with ultrastructural findings in myocardial and hepatic cell nuclei after in vivo exposure to adriamycin. Subcellular studies by Lambertenghi-Deliliers (17) in adriamycin-treated mice revealed a sequence of significant changes in hepatic cell nucleoli characterized by fragmentation and segregation of nucleolar components. These nucleolar lesions appeared in all parts of the liver lobule, were dose dependent, and were interpreted to be the result of disturbed nucleolar DNA and RNA synthesis induced by adriamycin. Buja et al (18,19) reported unusual nuclear alterations in myocardial cells from patients receiving daunorubicin and adriamycin and in explants of adult African green monkey myocardium cultured with daunorubicin. The nuclear changes were similar in both patients and myocardial explants and consisted of replacement of chromatin by thick and intermediate fibers and thin filaments. They postulated that the nuclear alterations were the result of unravelling, uncoiling, and disaggregation of chromatin induced by adriamycin's intercalating action on DNA.

These studies correlate well with recent findings of intense microscopic fluorescence in nuclei of various organs from adriamycin-treated hamsters (20). Fluorescence was evident in all tissues sampled at 0.5, 15, and 60 minutes after injection of adriamycin. Early cytoplasmic fluorescence, evident in only kid-

[5]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6; daunomycin.

ney and liver cells, dissipated and became concentrated in nuclei by 60 minutes.

Reports of toxic effects of adriamycin in animals and man indicate that tissues of eight different organ systems are adversely affected by this agent. Toxic symptoms involving the digestive, hematopoietic, lymphoreticular, and reproductive organ systems are, in general, characteristic of the action of the majority of antineoplastic agents. Myelosuppression, digestive disturbances, stomatitis, and alopecia are the most frequently occurring adverse effects in patients receiving adriamycin (2). Leukopenia and thrombocytopenia may be severe and thus dose limiting. Adverse effects on cardiovascular, urinary, skeletal, and integumentary systems in various species are rather unique alterations related to the administration of the adriamycin class of agents and will be considered in detail in this report. Of these infrequently occurring toxic effects, to date only the cardiovascular toxicity, which occurs more frequently if the total dose exceeds 550 mg/m^2, appears to be of major concern in man.

Cardiovascular Toxic Effects

The cardiovascular toxicity of adriamycin has both peripheral and central adverse effects. The peripheral toxicity is related to pathologic alterations at intravenous ((iv) injection sites where extravasation of drug invariably results in severe thrombophlebitis, angiitis, and cellulitis with necrosis and sloughing of tissue. This localized perivascular toxicity has been observed in both experimental animals and man receiving iv doses of adriamycin.

The central toxicity of adriamycin on the myocardium is the major total dose-limiting toxic effect in patients. In 1967, Tan et al (21) reported on the cardiotoxic effects of daunorubicin and recommended that total doses per patient not exceed 25 mg/kg (925 mg/m^2). Autopsy studies on eight of 14 of their patients who died revealed no consistent histopathologic changes in the myocardium. Subsequent studies by a number of investigators have led to the conclusion that the adriamycin-induced cardiotoxicity appears in two distinct forms, ie, electrocardiographic (ECG) and cardiomyopathic. The ECG form is characterized by transient alterations which are not dose or schedule dependent and occur within a few days after administration of the drug. The cardiomyopathic form is characterized by ventricular failure and congestion which may be irreversible and fatal. This arbitrary classification does not preclude the fact that ECG changes may occur before and in conjunction with the cardiomyopathic form. The unique aspects of this potentially lethal cardiotoxicity which is manifested by delayed insidious, and irreversible characteristics have stimulated many investigators to attempt to elucidate its pathogenetic mechanisms. A number of animal models have been characterized to various degrees in an attempt to contribute to a better understanding of the adriamycin-induced cardiotoxicity (table 1). An animal model would be valuable in testing analogs of adriamycin for cardiotoxic potential and for testing antidotal procedures to prevent or nullify the development of the cardiomyopathy. The "ideal" experimental animal model for cardiotoxicity should:

(*a*) exhibit a cardiomyopathy which progresses to congestive heart failure;
(*b*) have measurable clinicopathologic parameters with reasonable endpoint(s);
(*c*) exhibit total dose-related cardiomyopathy;
(*d*) be a result of multiple iv injections;
(*e*) exhibit pharmacokinetics similar to those in man;
(*f*) have a high, reproducible degree of incidence (> 50%);
(*g*) exhibit extracardiac toxicity similar to that in man; and
(*h*) be manageable with respect to size, temperament, care, housing, and availability of tissues and body fluids for study.

Of the possible animal models under investigation (table 1), only the rabbit and monkey appear to develop a dose-related cardiomyopathy with congestive heart failure. The results of studies from a number of laboratories have led to a detailed characterization of the cardiomyopathic syndrome in the rabbit (22-26). Further investigations are needed to define more fully, on a comparative basis, the clinicopathologic effects of adriamycin in the monkey and in other proposed animal models. Since the majority of the cardiotoxicity studies have involved the rabbit model, this species will serve as a prototype for the following discussions of the clinicopathologic effects of adriamycin administration.

The iv administration of multiple, low doses of adriamycin to rabbits results in a cardiotoxic syndrome characterized by generalized subcutaneous edema (anasarca), ascites, pleural effusions, hydropericardium, hepatomegaly, cardiomegaly, biventricular failure, and morphologic and biochemical alterations of the myocardium. Passive congestion of body tissues is evident in a number of cardiomyopathic rabbits with development of a swollen, edematous liver that has a characteristic "nutmeg" appearance grossly. Histopathologic examination reveals mild to severe centrolobular congestion and necrosis (fig 1).

Analyses of serum from adriamycin-treated rabbits have revealed a number of significant alterations (tables 2-4). Although serum enzyme concentrations (ie, serum glutamic oxaloacetic transaminase [SGOT], lactic dehydrogenase [LDH], creatine phosphokinase [CPK]) are increased, their significance along with ECG changes in the rabbit (27) in the pathogenesis of the syndrome remains to be elucidated. Aubert et al (28) concluded that no absolute parallelism existed between variations of serum CPK and SGOT levels and myocardial lesions occurring in daunorubicin-treated rabbits. These findings are compatible with those in man which indicate

TABLE 1.—Various dose schedules used for induction of experimental anthracycline cardiomyopathies in animal models

Species	Dose schedule (mg/kg/day)	Route of administration	Investigator(s)	Ref No.
Rat	4-8, × 5-6 days	Ip	Cargill et al	31
	2-8, × 6 days	Ip	Ainger et al	33
Rabbit	0.25-1, × 90 days	Iv	Maral et al	22
	0.8, × 3 days q15d	Iv	Bertazzoli et al	36
	0.7, 3 × wk	Iv	Young et al	25
	0.8-1.25, 2-3 × wk	Iv	Jaenke	24
	0.5-1, × 3-5 days q15d	Iv	Bertazzoli et al	37
	3.2, q21d	Iv	Young et al	52
Mouse	20, × 1 day	Ip	Liss et al	32
			Rosenoff et al	39
Monkey	3-6, q28d	Iv	Denine et al	38
Hamster	1-100, × 1-4 days	Iv, ip	Farmar et al	35

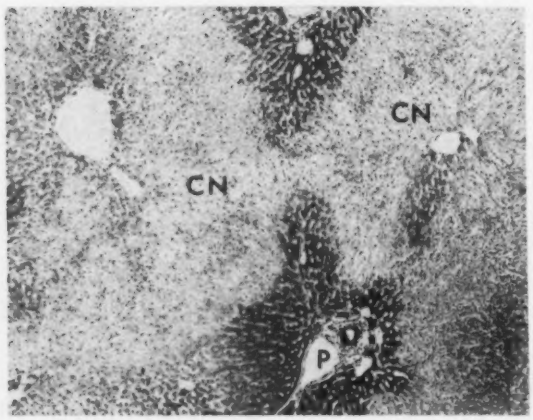

FIGURE 1.—Section of liver from an adriamycin-treated rabbit receiving a total dose of 175 mg/m². Note extensive areas of centrolobular coagulation necrosis (CN); viable hepatic cells surrounding portal areas (P). (H & E stain, × 49)

that transient ECG changes (2) and serum enzyme (CPK, SGOT, LDH) alterations (29, 30) are not ideal predictors for impending congestive heart failure. Experimental studies in the rat model indicate that ECG alterations are characteristic of a delayed effect of multiple injections of daunorubicin (31).

The daunorubicin/adriamycin-induced morphologic alterations in myocardium have been well characterized in both experimental animals and man (18,24-26,30,32-39). Cardiomegaly with biventricular dilatation and occasional thinning of ventricular free walls are usually characteristic findings. The formation of mural thrombi reported to occur in man (18,30,34,40) does not appear to be a characteristic finding in animals. Histopathologically, the myocardial lesions in the rabbit are characterized by edema, vacuolization of myocytes, myocytolysis (myocyte degeneration and necrosis), atrophy of myocytes (figs 2 and 3), and replacement fibrosis. These changes, which occur predominantly in the ventricles in a multifocal distribution, are occasionally confluent and are evident grossly as pale areas. A complete topographic distribution study of the adriamycin-induced microscopic myocardial lesions remains to be reported. Our studies in the rabbit indicate that the initial and subsequently the most severe changes occur in close proximity to major coronary arterial branches and arterioles. Extensive perivascular fibrosis is a common finding in a number of cardiomyopathic rabbits. The lesions are predominantly in an intramural location, although subendocardial areas and papillary muscles may be severely affected. The lesions appear to occur with some degree of consistency at the apex of the heart, in the septum, and near the junction of the ventricular free walls with the septum. A report by Jaenke (24) indicates that a consistent predisposition for subendocardial, subepicardial, or perivascular locations was not evident in studies of adriamycin- and daunorubicin-induced cardiomyopathy in rabbits. Ainger et al (33) observed a perivascular distribution of lesions and fatty replacement of the myocardium in a 12-year-old patient with leukemia who had fatal cardiotoxic symptoms after daunorubicin treatment. Rats treated with single iv doses of daunorubicin have ultrastructural evidence of damaged myocardial endothelial cells characterized by swelling and partial occlusion of the capillary lumina (41). Histopathologic changes throughout the myocardium have been reported in daunorubicin-treated patients although an increased frequen-

TABLE 2.—Serum electrolytes in nine adriamycin-treated and eight control rabbits

Group	No. of rabbits	Mean ± SE					
		Calcium	Magnesium	Phosphorus	Sodium	Potassium	Chloride
		mg/100 ml			mEq/liter		
Cardiomyopathy	4	13.3±0.8	2.7±0.3	8.2±2.9	130±3*	15.1±4.8	93±3
No cardiomyopathy (rabbits killed prior to onset)	5	12.8±1.1	2.1±0.2*	4.8±0.4*	141±4	8.5±0.8	101±3
Controls	8	14.1±0.2	2.7±0.1	6.8±0.4	141±2	8.9±0.5	97±2

*$P < 0.05$.

TABLE 3.—Serum enzymes of nine adriamycin-treated and eight control rabbits

Group	No. of rabbits	Mean ± SE				
		LDH	ALP*	SGOT	SGPT	CPK
		(IU)			(Dade units)	(units)
Cardiomyopathy	4	560±74†	30±6†	219±101	48±10	1882±855
No cardiomyopathy (rabbits killed prior to onset)	5	210±25	55±12†	82±17	64±11	493±130
Controls	8	226±21	98±11	65±7	40±5	374±64

*ALP = alkaline phosphatase.
†$P < 0.05$.

TABLE 4.—Serum values of nine adriamycin-treated and eight control rabbits

Group	No. of rabbits	Mean ± SE				
		Blood urea nitrogen	Uric acid	Cholesterol	Triglycerides	Total lipids
		mg/100 ml)				
Cardiomyopathy	4	76±9*	3.8±1.5*	222±35*	348±60	768±111*
No cardiomyopathy (rabbits killed prior to onset)	5	26±2*	0.9±0.2	192±73	386±251	1045±406*
Controls	8	23±1	0.6±0.1	64±6	182±73	334±29

*$P < 0.05$.

cy of subendocardial distribution of lesions was observed (19). Studies of myocardia from patients treated with daunorubicin have indicated microscopic damage to intrinsic cardiac neurones (42). Reports by other investigators studying animal and human myocardia after treatment with adriamycin or daunorubicin have failed to confirm this finding of neuronal lesions (18,24). A histofluorimetric study by Mazzanti et al (43) utilizing sections of intestines from daunorubicin-treated guinea pigs and rats indicates that daunorubicin does localize in the nerve cells of the Auerbach, but not the Meissner's plexuses. Further detailed studies are required to characterize fully the neuromuscular and perivascular distribution of adriamycin/daunorubicin-induced changes within the heart.

Occasionally inflammatory infiltrates in the myocardium are evident microscopically, presumably in response to the extensive degeneration and necrosis of myocytes. A number of nonvacuolated myocytes appear shrunken and atrophic (fig 2). Interstitial spaces are occupied by edematous fluid, adipocytes, and collagen fibers. Findings in our laboratory indicate that the water content of heart muscle from cardiomyopathic rabbits is significantly greater than that from matched saline-treated controls (26). In this respect, findings of "cardiomegaly" and "increased heart weights" in adriamycin-treated rabbits must be interpreted cautiously regarding indications of myocardial hypertrophy.

Detailed ultrastructural evaluations of affected myocardium from patients and animals receiving either adriamycin or daunorubicin indicate that nearly all of the subcellular organelles of the cardiac myocyte are altered (19,24,26,30,41). Myocardium from cardiotoxic rabbits exhibits myofibrillar, sarcotubular, and mitochondrial changes (figs 4-6). Dilatation of the sarcoplasmic reticulum and transverse tubular system appears to be one of the earlier changes and corresponds to vacuolation of myocytes evident at the light microscopic level (24,26,30). The distentions of the sarcotubular system may become so extensive they will displace and/or obliterate adjacent myofibrils and mitochondria (figs 5-7).

Myofibrillar degenerative changes include loss of parallel orientation, disruption of Z-band registry, spreading or widening of Z-band material, fragmentation into component sarcomeres, lysis, paucity of thick (myosin) and thin (actin) filaments, disorientation of remaining myofilaments (cross weaving), and eventual fibrous replacement (figs 6 and 8). Numerous affected cells appear to be nearly void of myofibrillar organelles (figs 4 and 5). Quantitative electron microscopic studies indicate that myofibrils occupy approximately 48% of the cell volume of a normal cardiac myocyte (44).

Mitochondrial damage appears to be a consistent finding in degenerating myocytes. Early changes appear to be characterized by swelling, separation of cristae, and outer mitochondrial membranes with increased electron density to peripheral margins (figs 5 and 6). Multilaminated concentric whorls or myelin figures are observed in intra- and peri-mitochondrial locations. Eventually mitochondria undergo shrinkage and condensation, and appear as small oval electron-dense inclusions a fraction of their original size (fig 6). Affected mitochondria have been reported to contain small spherical electron-dense granules which are interpreted as concentrations of cationic (ie, Ca^{++} and Mg^{++}) complexes (32,39). Hyperplasia of mitochondria with fusion of outer membranes has been reported to occur in the myocardium of daunorubicin-treated rats (33). Membranous whorls surrounding degenerating mitochondria have been observed in adriamycin-treated mice (39) and daunorubicin-treated rats (41).

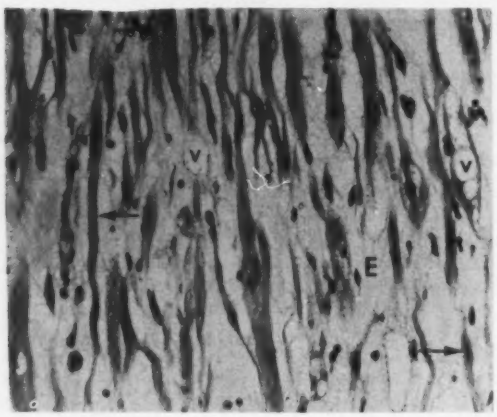

FIGURE 2.—Section of myocardium from an adriamycin-treated rabbit receiving a total dose of 175 mg/m². Note vacuolization of myocytes (V); interstitial edema (E); atrophy of remaining myocytes (arrows). (H & E stain, × 122)

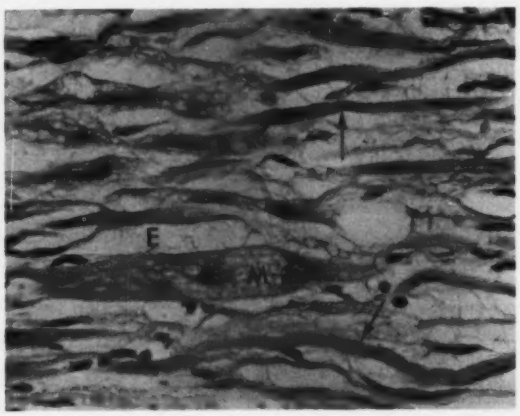

FIGURE 3.—Section of myocardium from an adriamycin-treated rabbit receiving a total dose of 175 mg/m². Note swelling and vacuolization of myocyte (M); adjacent myocytes appear atrophic (arrows); E = interstitial edema. (H & E stain, × 486)

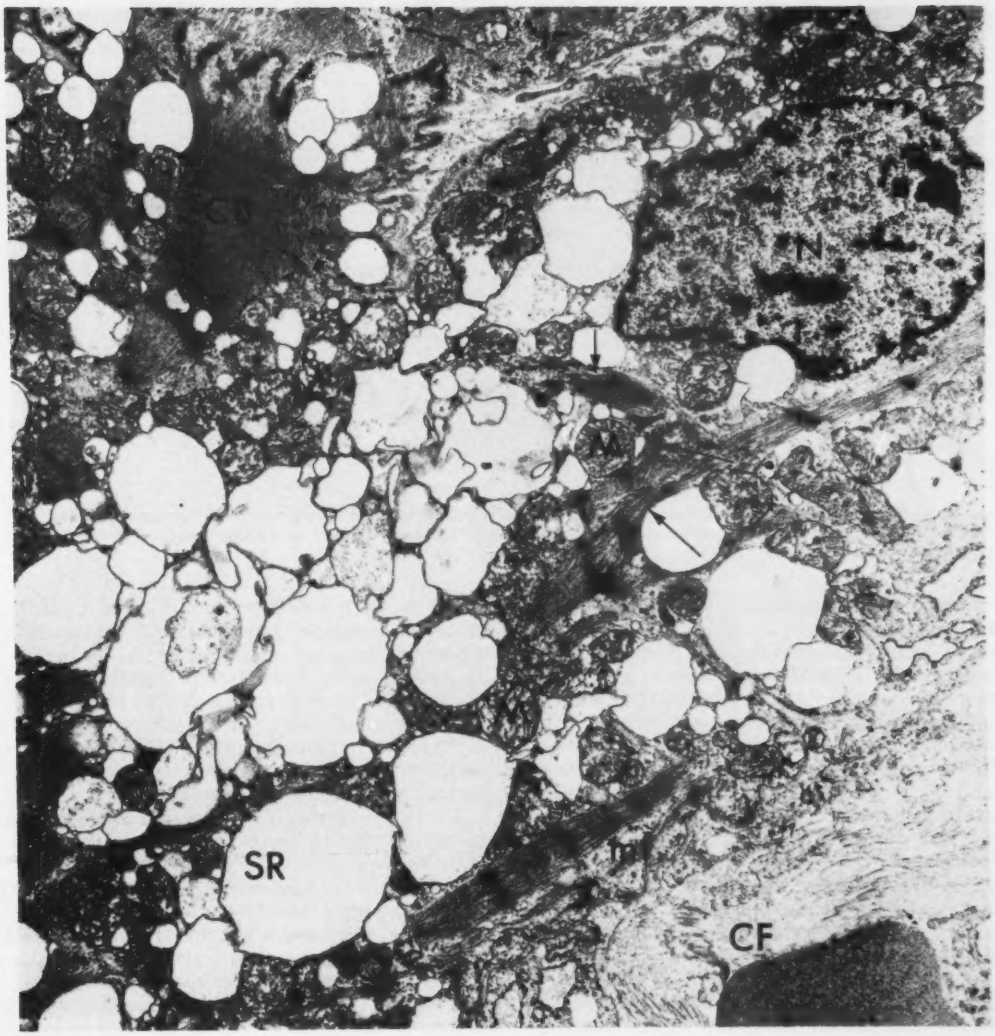

FIGURE 4.—Cardiac myocyte from an adriamycin-treated rabbit receiving a total dose of 400 mg/m². Note extensive vacuolization of sarcoplasmic reticulum (SR) and sparsity of myofibrils (mf). Myofilaments are sparse and appear occasionally in a cross-weaving arrangement (arrows). N = nucleus; M = mitochondria; CB = contraction bands; CF = collagen fibers. (Uranyl acetate and lead citrate, × 10,000)

The sarcoplasm of myocytes from adriamycin-treated rabbits is depleted of glycogen granules and frequently contains concentrations of subcellular membrane fragments, particles, and other debri (figs 6 and 8). Proliferation of collagen fibers is evident adjacent to the sarcolemma of degenerating myocytes (figs 4 and 7).

Nuclear alterations include pleomorphism with disorientation of chromatin. Chromatin alterations in daunorubicin-treated patients and monkey heart cultures have been described in detail by Buja et al (18,19). Evaluation of myocyte nuclei from treated animals has failed to reveal a nuclear change similar to that reported in patients. Further investigations are needed to evaluate fully the significance of these reported chromatin alterations in cardiac myocytes.

Biochemical correlates of the morphologic alterations in the myocardium have been reported in rabbits (26) and mice (32). Studies from our laboratory indicate that rabbits with adriamycin-induced cardiomyopathy have significantly elevated myocardial calcium and sodium concentrations compared to controls (26). These electrolyte elevations were evident

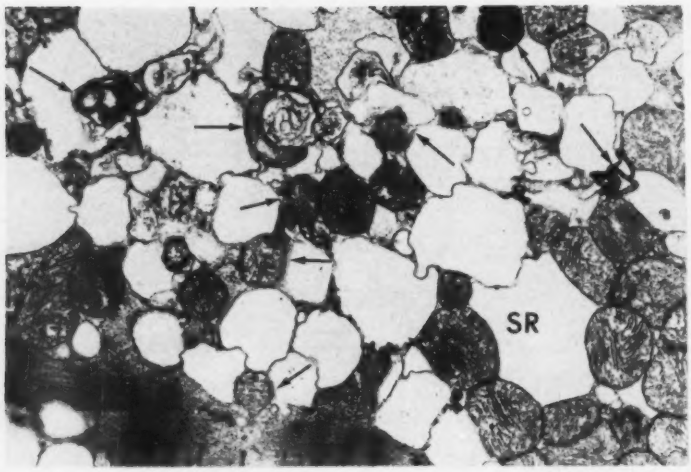

FIGURE 5.—Sarcoplasmic area from an adriamycin-treated rabbit receiving a total dose of 400 mg/m². Note distentions of sarcoplasmic reticulum (SR) and disintegration and condensation of outer mitochondrial membranes (arrows) with myelin whorls. CB = contraction bands. (Uranyl acetate and lead citrate, × 15,700)

in adriamycin-treated rabbits killed before cardiomyopathy developed and in those with terminal congestive heart failure. Therefore, calcium and sodium accumulation in the myocardium appears to precede and accompany the development of a histopathologic alteration. An adriamycin-induced alteration in membrane permeability would correlate well with the reported increased hydration of myocardium and distentions of the sarcotubular system. The cytotoxic effects of an excessive intracellular accumulation of calcium in cardiac myocytes are well known (45). The calcium overload results in a deleterious breakdown of myocardial adenosine triphosphate and creatine phosphate, creating a high-energy phosphate exhaustion. This pathogenic mechanism has been postulated to be of etiologic importance in a number of experimentally induced cardiomyopathies (45). The exact significance of myocardial calcium accumulation in the adriamycin-induced cardiotoxic syndrome remains to be fully elucidated. The calcium overload hypothesis would correlate well with the ultrastructural findings in adriamycin-treated patients (30) and mice (32,39) of electron-dense intramitochondrial granules interpreted as calcium salts. Recent findings indicate that adriamycin-treated mice also have cardiomyopathy with increased concentrations of myocardial calcium (39). Chronic adriamycin toxicity tests in dogs and rabbits by Bertazzoli et al (46) failed to produce cardiomyopathy. Analyses of myocardial electrolyte concentrations performed in dogs, but not rabbits, revealed a 33% reduction in calcium and no alterations of sodium, potassium, or phosphorus concentrations.

Additional biochemical studies indicate that adriamycin induces a delayed suppression of DNA synthesis in the myocardium of BDF_1 and C57Bl/6 mice which is temporally related to fatal toxicity (47). Other antineoplastic agents (ie, 5-FU) fail to show this pronounced depression of myocardial DNA synthesis. The specific source of the myocardial DNA (ie, myocytic—nuclear or mitochondrial; endothelial or fibroblastic) and the role of its suppression in the pathogenesis of the adriamycin-induced cardiomyopathy remain to be determined.

Further biochemical postulates of the mechanism of adriamycin cardiotoxicity include a proposed action on myocardial membrane enzymes (48). Calcium-stimulated adenosine triphosphatase (ATPase) and epinephrine-responsive adenyl cyclase systems have been proposed although the authors failed to indicate a positive or negative action or present experimental evidence to support their thesis. It is interesting to speculate that adriamycin may have a negative (inhibitory) effect on sodium-potassium ATPase activity similar to cardiac glycosides. This action would correlate well with the findings of sodium and calcium accumulation in the intoxicated myocardium. Although the pathogenesis of the cardiotoxicity induced by adriamycin has been the topic of numerous studies, considerable work is still required to elucidate fully its mechanism of action. The possible mechanisms of adriamycin cardiotoxicity are interference with *(a)* DNA-directed RNA synthesis; *(b)* mitochondrial function; *(c)* membrane permeability (ie, electrolytes, enzymes); and *(d)* neurovascular function.

The rabbit appears to be a workable model that has adriamycin pharmacokinetic similarities to man (49). Harris et al (50) have recently proposed a pharmacokinetic modeling of adriamycin in the rabbit. Adriamycin does appear to be more cardiotoxic than

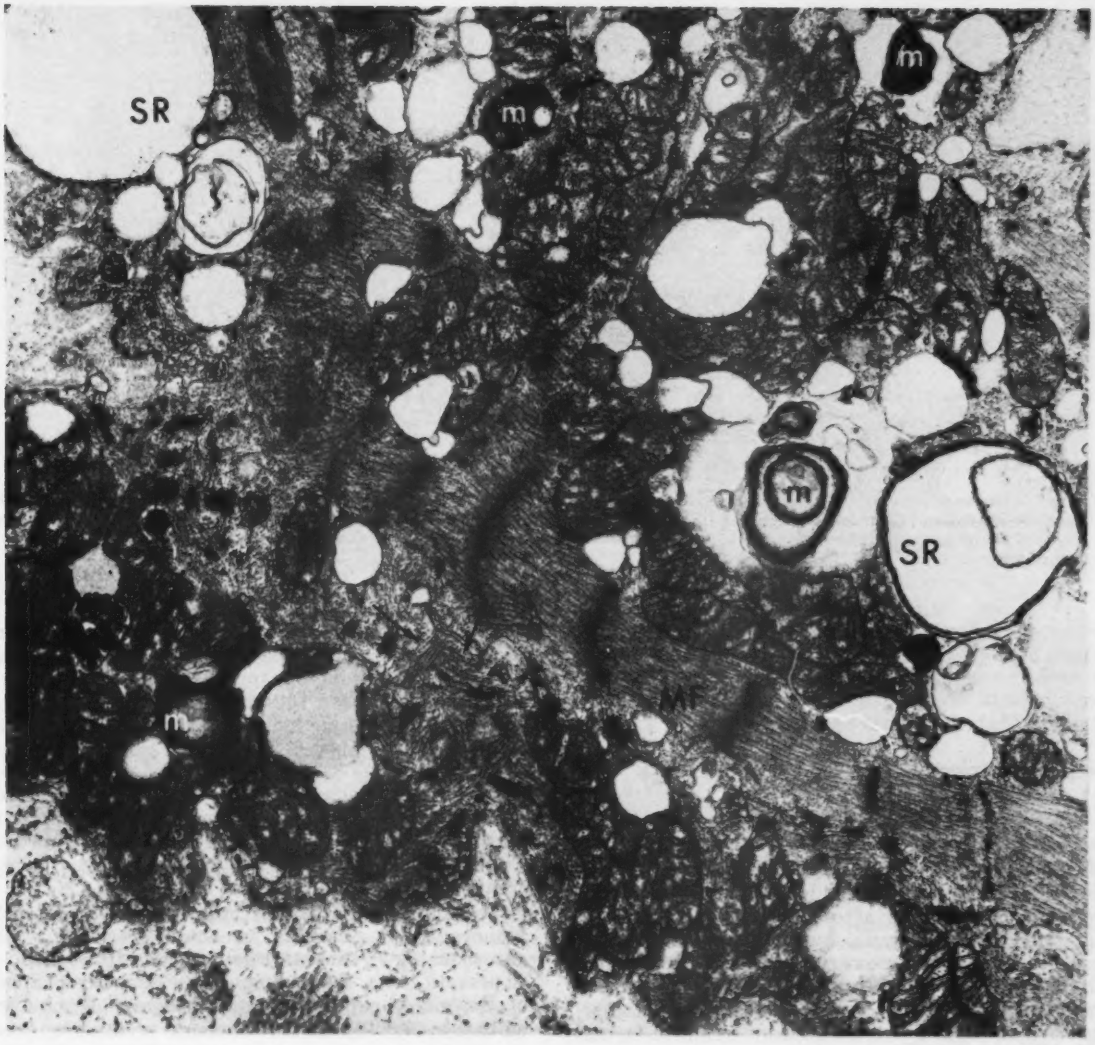

FIGURE 6.—Higher magnification of sarcoplasmic area from adriamycin-treated rabbit. Note vacuolization of sarcoplasmic reticulum (SR); degenerating mitochondria (m) with increased electron density and myelin whorls, and disarray of myofilaments with cross weaving (arrows). MF = myofibril. (Uranyl acetate and lead citrate, × 20,000)

daunorubicin in the rabbit which is contradictory to the findings reported in patients (51). Other potential animal models (ie, monkey, mouse, rat) warrant further characterization of clinicopathologic effects of adriamycin. A small experimental animal model is preferred because of the limited availability of synthetic adriamycin analogs and because experimental tumor systems in smaller animals may allow for a simultaneous determination of cardiotoxic and oncolytic activity (52). The rabbit model has been utilized recently by Eltringham et al (53) to test the toxic effects of combined radiation therapy and adriamycin on the heart. Preliminary results indicate that rabbits receiving the combined modality have myocardial lesions that are more severe than those observed in the single-treatment groups. Pericardial effusions, pericardial and myocardial fibrosis, and myocyte vacuolization and degeneration were prominent histopathologic changes in rabbits receiving 1600 rads of radiation and a cumulative dose of 167 mg/m^2 of adriamycin. Ongoing studies by these investigators are designed to determine if this com-

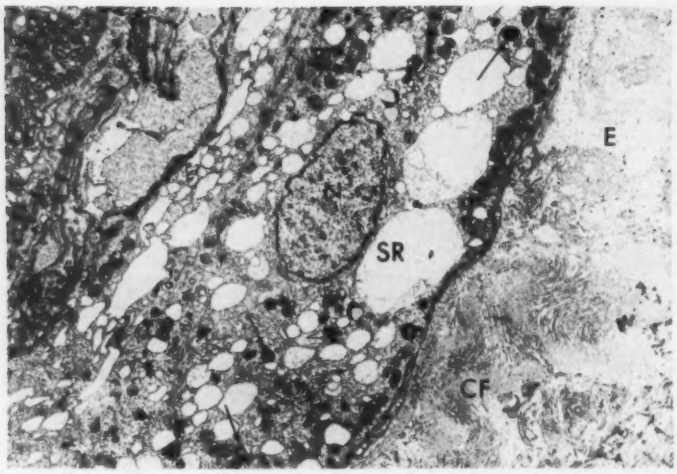

FIGURE 7.—Cardiac myocyte from an adriamycin-treated rabbit killed after receiving a total cumulative dose of 400 mg/m² over a period of 4 mos. Note distentions of sarcoplasmic reticulum (SR), degenerating mitochrondria (arrows), and a striking paucity of myofibrillar elements (m). Adjacent intercellular space is occupied by edematous material (E) and collagen fibrils (CF). N = nucleus. (Uranyl acetate and lead citrate, × 4800)

bined cardiotoxic effect is synergistic or additive. These findings correlate well with a previous report in children of increased susceptibility to the cardiotoxic effects of adriamycin if incidental radiation therapy to the heart has been received (54).

Nephrotoxicity

The nephrotoxic characteristics of anthracyclines were first reported in 1967 (55). Single iv injections of daunorubicin (20 mg/kg) given to rats resulted in the development of a nephrotic syndrome which persisted up to 1 year after injection and was characterized by proteinuria, hyperlipemia, edema, hypoproteinemia, and glomerular damage. A detailed ultrastructural evaluation of these changes in glomeruli revealed loss of foot processes, vacuolar formation, thickening of the basement membranes, and the presence of filaments and cross-striated fibrils in epithelial cells (56). Further nephrotoxic studies using daunorubicin in rats (57) have revealed development of chronic glomerulonephritis associated with a secondary hyperparathyroidism, metastatic calcification, and hyperostotic bone disease. Additional findings included the presence of cytomegalic cells in renal tubular epithelium. Other studies in these same rats revealed the presence of renal neoplasms in six of 33 pathologically evaluable animals 1 year after daunorubicin injection. Five daunorubicin-treated female rats had neoplasms of the genital tract. No urogenital tumors were present in control rats. This carcinogenic potential of daunorubicin for the urogenital system has not yet been reported in other species. Bertazzoli et al (58) observed a high incidence of mammary tumors in adriamycin- and daunorubicin-treated rats. Mean induction times for daunorubicin- and adriamycin-induced neoplasia were 121 and 223 days respectively in 20 of 50 rats. No neoplasms were found in 25 control animals. Mammary neoplasms induced by daunorubicin were mostly adenocarcinomas, whereas adriamycin tumors were mainly fibroadenomas.

We have found nephrotic syndrome-like effects with iv administration of adriamycin to rabbits. The syndrome is characterized by elevations in serum cholesterol, triglycerides, total lipids, uric acid, creatinine, and urea nitrogen (table 4), and by glomerulotubular damage. Histopathologically affected kidneys have extensive vacuolar destruction of glomeruli (fig 9), tubular degeneration with associated protein casts, thickening of basement membranes of Bowman's capsule, and cytomegaly of tubular epithelia (fig 10). Ultrastructural evaluation revealed that vacuolization begins within cytoplasmic areas of visceral and parietal epithelial cells of the glomerulus (fig 11). Coalescence of the vacuoles with obliteration of the glomerular tuft (fig 12) occurs as the lesion progresses in severity. Cytomegaly is vividly apparent ultrastructurally (fig 13) with sparse nuclear chromatin at the periphery and central aggregations present in a mosaic form. Other investigators have observed nephrotoxic effects of daunorubicin and adriamycin in rabbits (23,36,46).

Bertazzoli et al (46) reported tubulonephrotic changes in a group of rabbits treated with high doses in a chronic toxicology study. Azotemia was evident in intermediate- and high-dose groups, although urinalyses did not reveal any changes. Dogs utilized similarly in these chronic toxicity studies did not have altered renal function tests or histo-

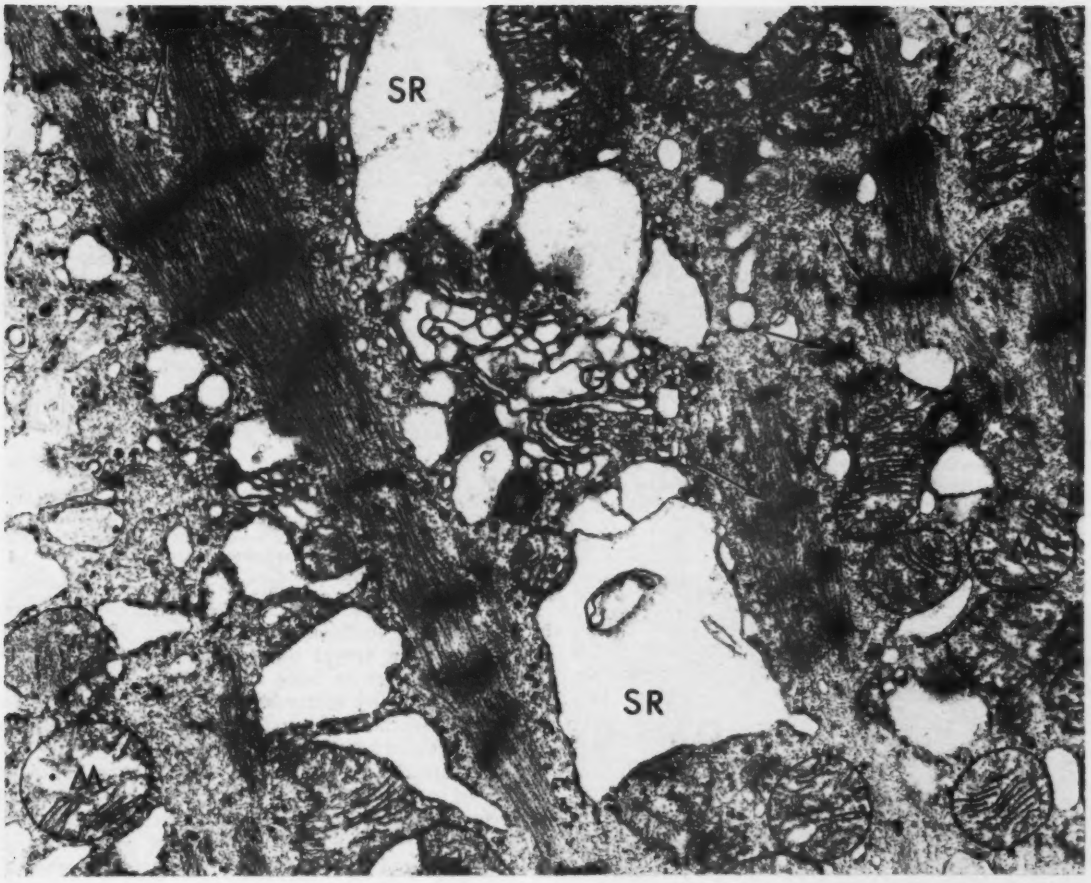

FIGURE 8.—Sarcoplasmic area of an adriamycin-treated rabbit receiving a total dose of 400 mg/m². Note distentions of sarcoplasmic reticulum (SR) and Golgi area (G) with fragmentation and lysis of myofibrils. Z-band material is dispersed and fragmented (arrows) among myofilaments. M = mitochondria. (Uranyl acetate and lead citrate, ×20,400)

pathologic changes in the kidneys. Reports of renal complications in patients have been infrequent. A report by Bonadonna et al (1) on the clinical evaluation of adriamycin indicated that no abnormal renal function was observed. Bernard et al (23) reported that two patients given daunorubicin therapy had renal tubular changes. In one patient a Fanconi's syndrome-like condition developed that was associated with hypocalcemia and clinical signs. The renal disturbances regressed after a period of complete remission from leukemia. Another patient had acute tubular nephritis with anuria. These renal lesions also regressed in time. Therefore, it appears from reported studies that the rat and rabbit are uniquely sensitive to the nephrotoxic effects of adriamycin. Further investigations in other experimental animals are needed to determine the extent of this susceptibility to adriamycin-induced nephrotoxicity.

It is interesting to speculate that the tetracycline portion of the adriamycin molecule (fig 14) may be responsible for the nephrotoxic effects in animals. The adverse effects of tetracycline-related drugs and tetracycline degradation products on man and animal kidneys have been the topic of a number of reports (59-63). Recent distribution studies utilizing a radioimmunoassay technique in rabbits indicate that the kidney has the highest concentration of adriamycin equivalents among eight different tissues analyzed 24 hours after injection (64).

Skeletal System Toxic Effects

Previous studies of low-dose, long-term administration of adriamycin to rabbits suggest a cytotoxic action on growing bone (65,66). It is well known that adriamycin is one of the most efficacious single chemotherapeutic agents against bone sarcomas

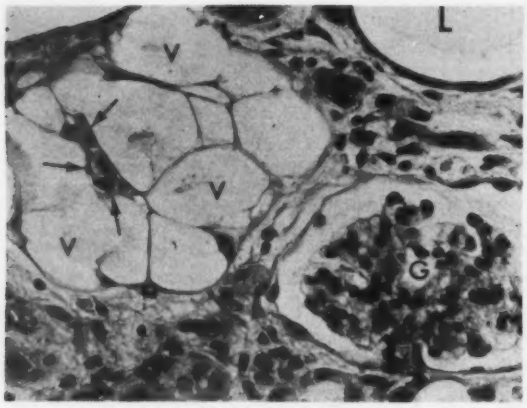

FIGURE 9.—Section of renal cortex from an adriamycin-treated rabbit receiving a total dose of 210 mg/m². More normal appearing glomerulus (G) is adjacent to a glomerulus destroyed by vacuolization (V). Only the few mesangial cells remain (arrows). L = lumen of dilated tubule. (H & E stain, × 486)

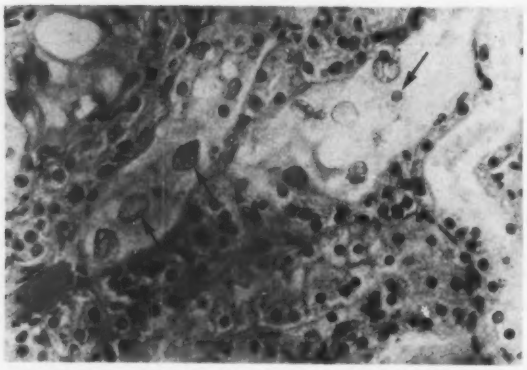

FIGURE 10.—Section of renal tubules from an adriamycin-treated rabbit. Note nuclear pleomorphism and cytomegaly (arrows). (H & E stain, × 486)

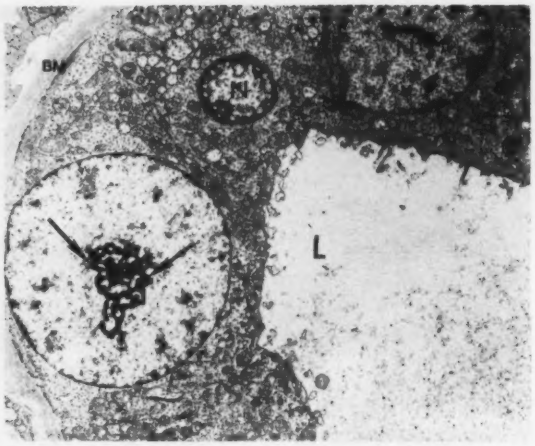

FIGURE 11.—Renal tubule from an adriamycin-treated rabbit. Note cytomegaly with sparsity of nuclear chromatin and mosaic central aggregates (arrows). BM = basement membrane; L = lumen; N = nucleus. (Uranyl acetate and lead citrate, × 6300)

(2,67,68). Adriamycin has been successfully used as cyclic adjuvant therapy to the high-dose methotrexate-citrovorum factor rescue therapy for childhood osteosarcomas (69).

Rabbits receiving adriamycin have physiochemical and morphologic alterations of chondro-osseous tissues which are most pronounced at the epiphyseal-metaphyseal areas of long bones. Epiphyseal cartilage plates are thin and dysplastic with deranged growth zones (figs 15 and 16). Metaphyseal areas are deficient in trabeculae, osteoblasts, and osteoclasts. Bones are significantly less dense than those from age-matched saline controls. Thin cortices with large irregular spaces are evident microscopically. These findings suggest that adriamycin produces a retardation of chondro-osteogenesis in growing rabbits. Bertazzoli et al (46) reported that rabbits used in chronic toxicity tests of adriamycin exhibited an arrest or reduction of body growth. This deficiency in bone maturation must be interpreted critically with respect to chronic debilitation and development of renal lesions in treated animals. Renal osteodystrophy is a well-characterized syndrome in a number of species. The osteopenia observed in adriamycin-treated rabbits does not readily fit the classifications of osteoporosis, osteomalacia, or fibrous osteodystrophy. The paucity of osteoblasts and the significant decreases in serum alkaline phosphatase (table 3) suggest an impairment of bone formation. These findings, along with the lack of osteoclastic activity, indicate a decreased production of well-differentiated osteogenic cells. Increased numbers of undifferentiated mesenchymal cells appear interposed between trabecular fragments. Perhaps adriamycin blocks the maturation of bone-remodeling cells, leading to an apparent overproduction of osteoprogenitor cells. This type of cytotoxic action on bone cells has been proposed for mithramycin, another antitumor antibiotic produced by *Streptomyces* (70). Further studies involving in vitro short-term cultures of adriamycin with fresh cortical bone powder revealed the presence of characteristic fluorescent binding to undecalcified, but not to decalcified, bone. These results correlate well with recent findings that suggest adriamycin readily complexes or chelates with metal cations (71). It is hypothesized that adriamycin adversely affects both the inorganic and organic components of bone with inhibition of bone-cell production, inhibition of synthesis and maturation of matrix constituents, and interference with crystal formation by complexing with inorganic (cationic) fractions.

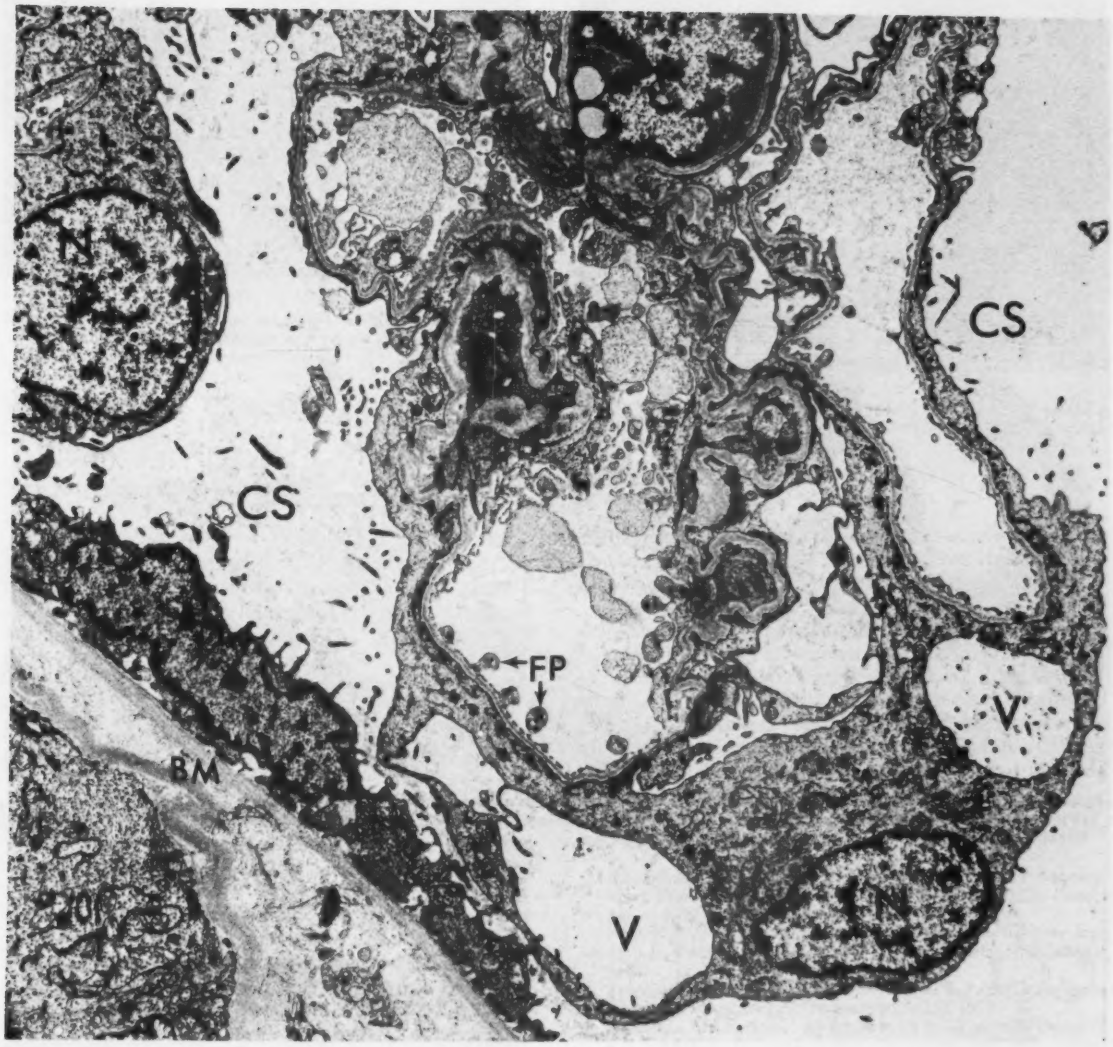

FIGURE 12.—Glomerulus from an adriamycin-treated rabbit. Note vacuoles (V) within cytoplasm of visceral epithelial cell of glomerulus. Basement membrane (BM) appears thickened. CS = capsular space; N = nucleus; FP = foot processes. (Uranyl acetate and lead citrate, × 7600)

Adriamycin does have structural and chemical similarities to other bone-seeking agents (fig 14). Alizarin red is a dihydroxy-anthraquinone that has routinely been used as a stain for chondro-osseous tissues in a number of teratogenic and experimental studies. Adriamycin readily stains freshly ground (70μ thick) undecalcified sections of bone.

The bone-seeking properties of tetracyclines are well known. Previous studies have shown that tetracycline inhibits both bone growth (72) and amorphous-crystalline transformation of calcium phosphate (73). Adriamycin and tetracycline may compete for a number of similar receptor sites in various tissues. Prior treatment with oxytetracycline in daunorubicin-treated mice markedly reduced acute toxicity (74) and altered the pharmacokinetic distribution of daunorubicin (75). Further detailed investigations are required to characterize these adriamycin-bone interactions in other species including man. Perhaps adriamycin is sequestered at the level of the hydroxyapatite crystal in bone and released over a long period of time.

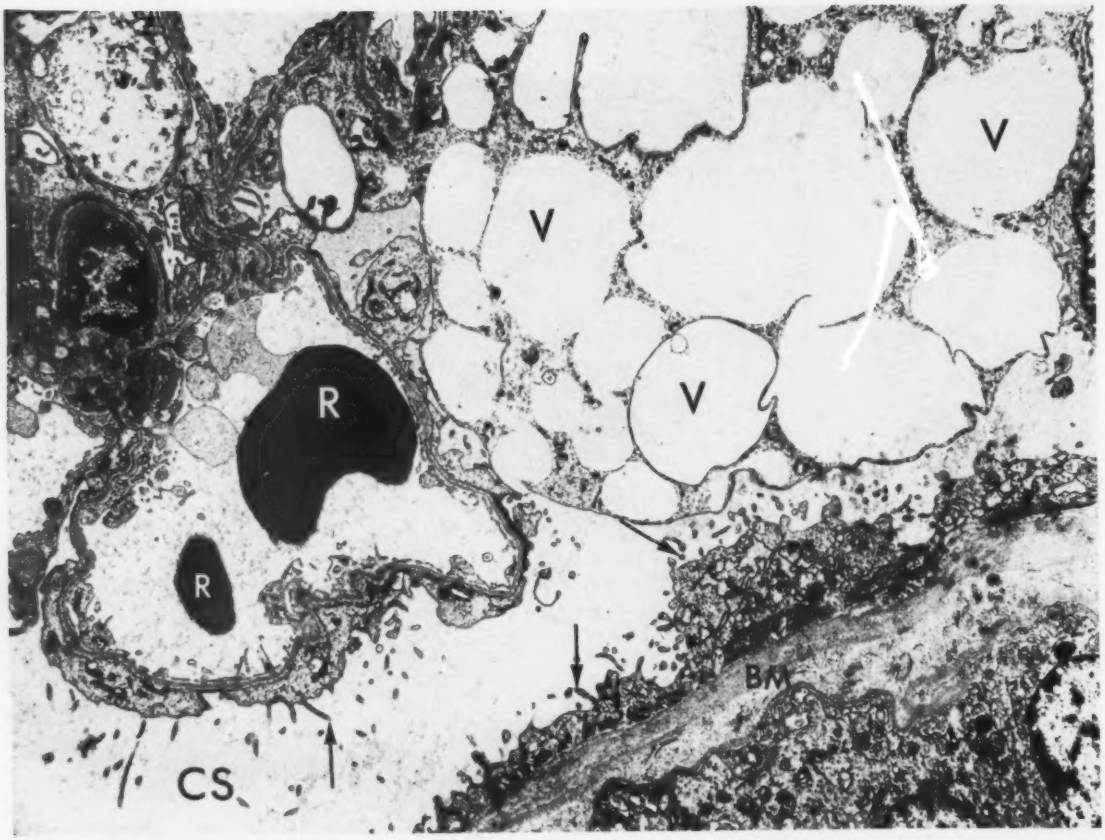

FIGURE 13.—Glomerulus from an adriamycin-treated rabbit. Note extensive vacuolization of glomerulus with coalescence of many vacuoles (V). Basement membrane (BM) appears thickened. R = red blood cells; CS = capsular space; arrows = microvilli. (Uranyl acetate and lead citrate, × 6250)

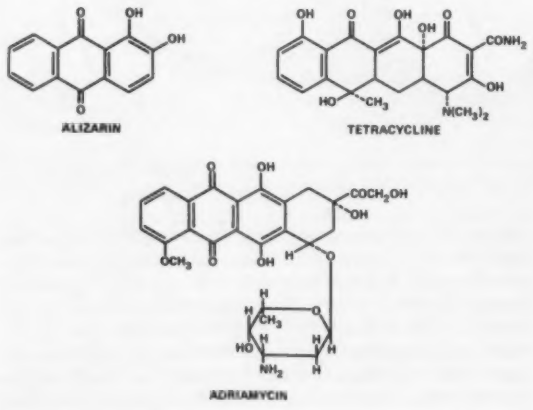

FIGURE 14.—Structural comparison of alizarin, tetracycline, and adriamycin.

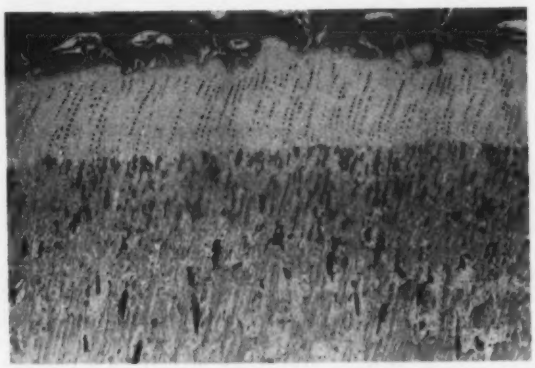

FIGURE 15.—Section of proximal epiphysis of tibia from saline-treated control rabbit. Note the order of endochondral ossification. (H & E stain, × 46)

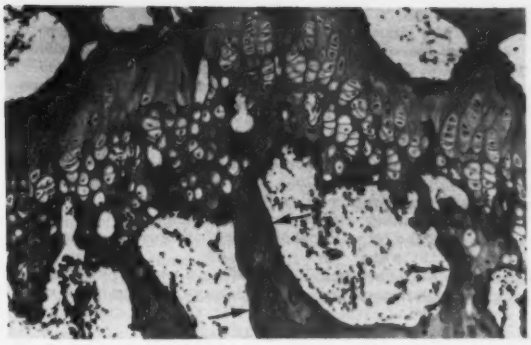

FIGURE 16.—Section of proximal epiphysis of tibia from an adriamycin-treated rabbit. Note dysplasia of cartilage plate with disarray of chondrocytes. Abnormally formed trabeculae are devoid of adjacent osteoblastic or osteoclastic activity (arrows). (H & E stain, × 90)

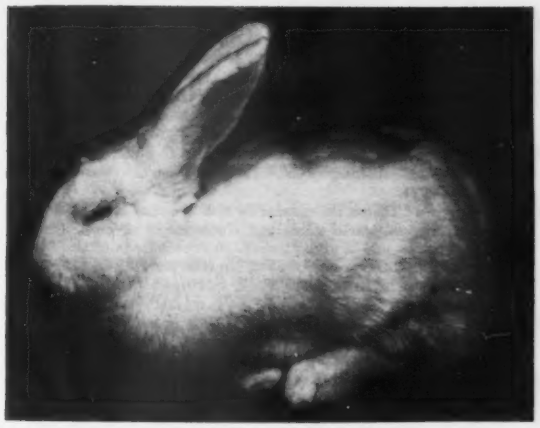

FIGURE 17.—Rabbit treated with multiple iv doses of adriamycin over a period of 4 mos. Note the debilitated appearance with mid-dorsal alopecia.

Integumentary Toxic Effects

Alopecia is the most frequent toxic effect of adriamycin administration. It has been reported to occur in 85%-100% of patients treated (2). Adriamycin administration does produce alopecia in animals on occasion, although it is seldom generalized in nature (fig 17).

Additional integumentary changes include hyperpigmentation, cellulitis due to extravasation at the injection site, and allergic reactions. In patients treated with adriamycin a hypermelanotic action has been reported to occur in the areas of nails, nail beds, dermal creases, palms, and phalanges (76,77). This adverse effect appears to be more frequent in blacks than whites. Bertazzoli et al (44) reported hyperpigmentation or "melanosis" occurring in the skin and buccal mucosa of dogs involved in a chronic toxicity study of adriamycin. Pigmentation of the oral mucosa has been reported to occur in a patient exhibiting a hypermelanotic reaction (77). A biopsy of an affected area from one patient showed increased melanin deposition with hyperplasia of melanocytes in the epidermis. The pathogenesis of this infrequent hypermelanotic syndrome is unclear. Further experimental studies are required to elucidate the mechanism of this adverse action of adriamycin.

A recent report by Etcubanas et al (78) indicates that adriamycin may cause allergic skin reactions manifested by pruritis, erythema, or urticaria. They evaluated reactions in 60 children who had received adriamycin alone or in combination with other agents. Eighteen episodes of allergic reaction occurred in 13 of the children. Most were localized rashes although one child exhibited a nonfatal anaphylaxis shortly after a localized reaction occurred. In two patients, prior radiation therapy to a localized site appeared to predispose to a cutaneous reaction after adriamycin administration. The pathogenetic mechanisms involved in this allergic phenomenon have not been elucidated.

Other integumentary changes include a report of skin neoplasms induced by daunorubicin in mice (23). Subcutaneous (sc) injections resulted in the development of fibrosarcomas at the injection site. Oral or intraperitoneal administration to mice produced no carcinogenic effect. The findings of fibrosarcomas after sc injection should be interpreted cautiously as viral-induced neoplasia may be a key factor in these studies, regardless of the agent used.

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Some Observations on the Toxicity of Adriamycin (NSC-123127)[1,2]

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SUMMARY

Adriamycin acutely damages vital, highly proliferative cell renewal systems such as the epithelium of the intestinal tract and all hematopoietic organs. In this respect, it is like other antitumor drugs. Adriamycin, however, has unique long-term toxic effects on the kidney (rats, rabbits) and on the heart (man, rabbits) which are characterized by lesions that evolve steadily over long periods. Cardiac glycosides have not been found to block uptake of adriamycin by rat and rabbit hearts in vivo or to prevent myocardial lesions in rabbits. The drug is tumorigenic in rats and can induce malignant transformation in mouse fibroblasts.

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We shall deal briefly with those toxic effects of adriamycin which are commonly observed when intoxicating doses are given to laboratory animals. Such effects consist of selective damage and inhibition of mitotic activity in tissues that are highly proliferative in postfetal life. These tissues comprise the proliferative compartments of cell-renewal systems in which large numbers of new cells are produced unceasingly to replace daily losses of mature components. Cell-renewal systems that are vitally important to the survival of the host include the mucosal epithelium of the digestive tract, the hematopoietic tissues in bone marrow, and the lymphoid organs. Systems less vitally important include those of hair follicles and spermatogenic tissues in the testis.

Bertazzoli et al (1) have shown that in laboratory animals adriamycin causes damage and prolonged inhibition in each of the cell-renewal systems just mentioned. Prolonged treatment of dogs and rabbits with fatal doses caused hemorrhagic enterocolitis and bloody stools in dogs and caused most hematopoietic organs to atrophy. Thrombocytopenia and anemia were prominent in both species, and leukopenia was seen in dogs. Prolonged treatment with lower doses, permitting survival, provided evidence of disturbance in hair growth; patchy alopecia occurred in dogs. Severe atrophy of the spermatogenic tissues was seen in both species (1).

To illustrate further the kinds of damage that are involved in fatal intoxication, we shall describe briefly the toxic changes seen in female rats that were given single intravenous (iv) doses of adriamycin. As shown in figure 1, animals injected with 20 mg/kg (an amount greater than the median lethal dose) lived for at least 4 days. Before death, they had severe weight loss and diarrhea. Such animals died between 4 and 9 days after receiving adriamycin. Only scattered fatalities occurred between 1 and 4 weeks after injections of 10 mg/kg, but eventually all of the remaining animals died within less than 15 weeks. Most rats survived for at least 1 year after receiving 5 mg/kg (*see* below).

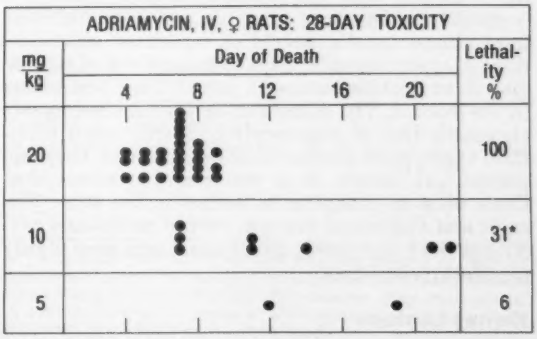

FIGURE 1.—Lethality in rats during the first 4 weeks after a single iv injection of adriamycin. The female CD rats (Charles River Breeding Laboratories, Wilmington, Mass) were 6-7 weeks old at time of injection. There were 26 in each of the groups that received 20 and 10 mg/kg; 36 received 5 mg/kg.

[1]Adriamycin: CAS reg. No. 23214-92-8.

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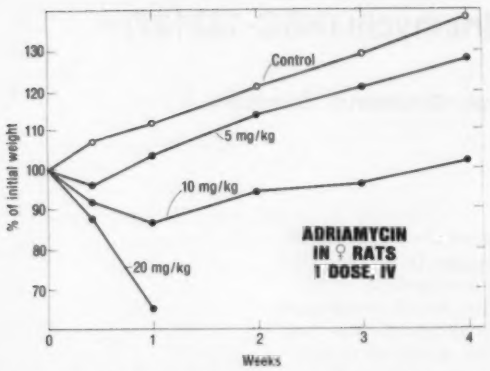

FIGURE 2.—Weight of rats during the first 4 weeks after a single iv injection of adriamycin. The values are averages for the rats in fig 1 that survived for at least 7 days after receiving 20 mg/kg and 28 days after the other doses. The 24 controls received 1 injection of 0.9% NaCl.

Figure 2 depicts the changes in body weight observed in these rats. It can be seen that after the initial loss of weight during the 1st week in animals receiving 10 mg/kg, there was some recovery. At various times after 5 weeks, however, the same rats showed a second period of steady weight loss that ended in marked cachexia and death. We have been unable to find specific lesions in these animals that might account for this greatly delayed lethal event nor have we observed such lethality in rats that received similar doses of daunorubicin.[6] One other change occurred in these animals that we have also not observed in rats that received daunorubicin, namely, a progressing asynergia of the hind legs indicative of damage in the sensory neuron tracts. This disturbance should be analyzed by detailed neuropathologic study.

Preterminal female rats, killed 7 days after receiving single doses of 20 mg/kg, had severe lesions in the intestine and the hematopoietic organs. All lymphoid tissues were atrophic and bone marrows were almost totally devoid of nucleated cells. Sections of colon revealed marked distortions of normal glandular architecture with hemorrhage and edema in the mucosa. The glandular epithelium had hyperchromatic foci of regeneration. In the small intestine, there were similar lesions although they appeared less severe. It is important to stress that there were *no* conspicuous lesions in the other thoracic and abdominal viscera; severe pathologic conditions were found only in organs containing highly proliferative tissues.

Kidney Lesions

In addition to its capacity to damage cell-renewal systems (a property shared with other antitumor agents), adriamycin has unique toxic actions which vary in expression in different species. The first of these to be discussed is kidney damage which so far has been reported only in rats and rabbits. Renal lesions appear to be the result of slowly progressing events which may extend over periods lasting months. Thus, we saw no changes by light microscopic study in the kidneys of the rats described above at 7 days after the acutely lethal dose of 20 mg/kg. In female rats given single iv doses of 5 mg/kg and killed 6-12 months later, however, we observed glomerular damage that consisted of focal thickening of the basement membrane and of the glomerular capsule. Protein casts were present in the tubules. Tubular architecture was altered by flattening and other distortions of the epithelium. These renal disturbances were similar to but less severe than those that occurred in rats treated with single doses of daunorubicin (2-4).

Recently, Bertazzoli et al (5) have described renal lesions in rabbits that were treated with adriamycin over a period of 6 months. We have also found severe renal lesions in nearly all rabbits that received multiple doses of adriamycin (see below for dose schedules and times of autopsy). The lesions were diffuse throughout the kidney cortex. Glomeruli were atrophic with fibrosis of capsule and distorted vacuolated capillary loops. There was marked loss of tubules and interstitial fibrosis. Many surviving tubules were abnormal and dilated; there were irregularities in epithelial-cell morphology. The lesions seemed to be progressive because there appeared to be active pathologic events that were evidenced by the presence of inflammatory cells.

Cardiotoxicity

The second of the unique pathologic effects of adriamycin is cardiotoxicity, a troublesome aspect of its use in patients. Adriamycin, like daunorubicin, can induce cardiac failure in man months after the first dose of the drug is given. The myocardium at the time of failure is severely damaged as shown by a loss of substantial fractions of the contractile sarcoplasm and by extensive fibrosis (6). A similar lesion has recently been reproduced in rabbits by long-term treatment with either adriamycin or daunorubicin (5,7,8).

Other researchers have shown that adriamycin can induce changes in cardiac function such as ECG arrhythmias in monkeys and hamsters (9), increases in coronary perfusion pressure in dog hearts (10), and negative inotropic effects in perfused rabbit hearts (11). The relation, however, between these short-term events, which appear to be largely reversible, and long-term cardiac pathologic events is not clear at present. We have searched for evidence of ECG changes in rabbits receiving long-term treatment with adriamycin. In some rabbits with extensive fibrotic replacement of contractile tissue, R voltages were decreased and T waves were flattened or inverted. In other rabbits with less severe

[6] Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

though definite lesions, we have observed no clearcut ECG abnormalities.

Recently we have found that acute myocardial lesions were rapidly induced by adriamycin in rabbits that received single iv doses of 10 or 20 mg/kg. These doses are about equal to the total cumulative amounts that are employed for induction of cardiomyopathy in animals treated over a long period of time. Rabbits given 20 mg/kg die within 5-24 hours after the injection. Shortly after the injection, such animals have sinus tachycardia, as shown by electrocardiography, but no other conspicuous arrhythmia. Within 1-2 hours after injection, they become increasingly dyspneic and weak. Eventually the heart rate slows and finally ceases. Autopsy immediately after death reveals abundant pericardial effusions. Histologic study of the myocardium shows a variety of severe pathologic changes. Edema is prominent in subepicardial regions. Interfibrillar hemorrhages are widespread thoughout the myocardium. Inflammatory foci and karyorrhectic nuclei are present. The latter are not cardiocyte nuclei since they have a capillary distribution. There are also scattered foci of sarcoplasmic vacuolization.

Since it has been reported that digitalis glycosides can block the negative inotropic effects of adriamycin in perfused rabbit hearts and can reverse the decrease in coronary flow and in cardiac output in dogs in vivo (11), we attempted to prevent the acute lesion by digitalizing rabbits with ouabain or digitoxin prior to the injection of the 20-mg/kg dose. In four animals so treated the lesion developed as rapidly and was as severe as in the nondigitalized controls.

It has also been reported that prior treatment of rats with strophanthin K greatly reduces the uptake of adriamycin by the heart in vivo, and that the same glycoside blocks entry of the drug into tissues of the perfused rabbit heart (11). We have seen, however, essentially no effect of prior digitalization with strophanthin K on the accumulation of adriamycin by rabbit hearts in vivo (table 1). The dose of strophanthin K used in these rabbits was sufficient to induce maximal digitalization as checked by electrocardiography. In other studies in rats we were equally unable to demonstrate any appreciable effect of digitoxin, ouabain, or strophanthin K on the cardiac uptake of adriamycin (table 2).

We have carried out a number of preliminary studies in rabbits with prolonged adriamycin treatment according to the various dose schedules described in table 3. Of the 13 survivors in groups A, B, and C given total doses of 11-15 mg/kg and killed 28 weeks after the first dose of adriamycin, 12 had chronic cardiac lesions. Among the 14 survivors in groups D and E, 11 had such lesions. In general, the pathologic effects were less advanced in the last two groups than in the first three. Presumably this was due to the earlier time of killing in groups D and E. If this is the correct explanation, it implies that the chronic lesion progresses steadily for long periods after cessation of treatment with the drug. In group B of table 2, the injection of digitoxin before each dose of adriamycin failed to prevent cardiomyopathy. The results obtained with the animals of group C suggest that fractionating doses may not prevent cardiomyopathy because each of these animals had cardiac abnormalities.

Incidentally, renal lesions were found in all but one of the 27 animals (table 3) that were autopsied. In general, these lesions were more severe in the first three groups than in groups D and E. Thus, the renal lesion in rabbits also appears to be a slowly progressing process.

Tumorigenicity

Several years ago, Bertazzoli et al (12) reported that single doses of either daunorubicin or adriamycin induced mammary tumors in rats. At about the same time, we described the induction of a variety of other kinds of malignant tumors in similar rats given single doses of daunorubicin (4). We have repeated our study of daunorubicin and simultaneously compared it with adriamycin. The results obtained are shown in table 4. Both agents have again been found to be tumorigenic. The data show the induction of a large number of benign mammary fibroadenomas and possibly an increased incidence of mammary adenocarcinomas over that observed in controls. The other malignant tumors in the treated animals included two unclassified subcutaneous tumors, one epidermoid carcinoma, and one liposarcoma in the groups receiving daunorubicin, and one adrenal carcinoma in the group receiving adriamycin. There are discrepancies among the results of the experiments just mentioned (the one reported by Bertazzoli et al and our two) with regard to the kinds of tumors produced by each of the agents in different groups of animals. Nevertheless, the three experiments are consistent in showing that the two anthracycline antibiotics are tumorigenic in rats.

TABLE 1.—Effect of strophanthin K on uptake of adriamycin by rabbit hearts in vivo*

Time after injection of adriamycin (min)	Amount (μg/g) of adriamycin in hearts after administering—	
	Strophanthin K	0.9% NaCl
16-17	31.3	31.9, 36.4
45-46	33.2	28.4
56-59	31.6	19.8, 18.4
238-250	15.2	11.7, 14.2

*Young adult male albino rabbits, weighing about 3 kg, were injected iv with either 150 μg/kg of strophanthin K (Kombetin, Boehringer-Mannheim) or 0.9% NaCl. Five minutes after the injection, each rabbit received 10 mg/kg of adriamycin iv. At various times thereafter, the rabbits were anesthetized iv with pentobarbital sodium and killed by exsanguination from the abdominal aorta. Each heart was rapidly removed, opened, blotted on filter paper, and weighed. The concentration of total fluorescent substance was determined by the procedure described by Bachur et al (15), with minor modifications, and was expressed in terms of equivalents of adriamycin. Each value in the table is the result from a single animal.

TABLE 2.—Effect of cardiac glycosides on uptake of adriamycin by rat hearts in vivo*

Cardiac glycoside injected 30 mins before adriamycin	Time after injection of adriamycin	Amount (μg/g) of adriamycin in hearts	
		Treated	Controls
Ouabain, ip	30 mins	18.6 ± 1.3	19.8 ± 1.7
Digitoxin, iv	30 mins	21.9 ± 2.4	22.7 ± 1.0
Strophanthin K, iv	30 mins	18.0 ± 0.9	19.7 ± 0.5
Strophanthin K, iv	24 hrs	7.0 ± 0.2	8.2 ± 1.2

*Young 7-week-old male CD rats (Charles River Breeding Laboratories, Wilmington, Mass) were injected in groups of 3 or 4 with either a cardiac glycoside (1 mg/kg) or 0.9% NaCl. Thirty mins later, each rat received adriamycin iv (10 mg/kg). At 30 mins or 24 hrs after adriamycin, the treated and control rats were etherized and killed by exsanguination from the abdominal aorta. Hearts were removed and analyzed as described in table 1. The glycosides used were ouabain USP (Lilly), digitoxin (Sigma), and strophanthin K (Kombetin, Boehringer-Mannheim). Each value in the table is the mean ± 1 SD for each group.

TABLE 3.—Schedules of injection in rabbits receiving repeated iv doses of adriamycin*

Group	Individual dose (mg/kg)	Total dose (mg/kg)	No. of rabbits surviving/ No. tested	Week of autopsy of survivors
A	3	13-15	4/6	28
B	3	11-14	4/6	28
C	0.6	14-15	5/6	28
D	6	18	4/5	13-18
E	6	18	10/16	7-15

*Groups of male albino rabbits, initially weighing 3-4 kg, received adriamycin as follows: group A, 3 mg/kg at 4-wk intervals; group B, 3 mg/kg 1 hr after digitoxin USP (30 μg/kg) at 4-wk intervals; group C, 0.6 mg/kg/day for 5 successive days at 4-wk intervals; group D, 6 mg/kg at 4-wk intervals; and group E, 6 mg/kg at 3-wk intervals. In groups A, B, and C, the adriamycin dose was decreased by ⅓ if the animal had failed to regain weight after the preceding injection.

TABLE 4.—Tumors in female rats receiving single iv doses of daunorubicin or adriamycin*

	No. of tumors appearing after 1 iv injection of—			
	Daunorubicin		Adriamycin (5 mg/kg)	0.9% NaCl (controls)
	10 mg/kg	5 mg/kg		
Incidence of animals with tumors†	12/16	15/18	15/17	5/20
Mammary tumors				
Fibroadenoma	6	12	16	1
Adenocarcinoma	5	2	3	2
Other malignant tumors	2	2	1	
Other benign tumors		1	1	2

*Seven-week-old female CD rats (Charles River Breeding Laboratories, Wilmington, Mass) received one iv injection of either daunorubicin, adriamycin, or 0.9% NaCl. The two groups receiving daunorubicin and the control group consisted initially of 20 rats; the group receiving adriamycin consisted of 25 rats.

†Some rats in each of the treated groups died unobserved; these were excluded from the data concerning the incidence of animals with tumors. The remaining animals were killed when observable tumors developed, when they appeared ill and were losing weight steadily, or at the end of 1 yr. At time of autopsy, samples were taken for microscopic study of kidneys, tumors, and any other organ that appeared grossly abnormal.

Further assessment of its potential for tumor induction must await additional study of adriamycin in other species observed for life-time periods. Casazza has already found that adriamycin, given subcutaneously in infant mice and rats, can induce fibrosarcomas at the sites of injection (unpublished observations cited in ref 13).

We have recently obtained additional evidence from in vitro studies that strongly suggests a carcinogenic potential of both adriamycin and daunorubicin. We are currently testing the capacity of both agents to induce malignant transformation in a cloned line of mouse fibroblasts. In previous work, such cells have been found to be transformed by polycyclic aromatic hydrocarbon carcinogens and by other potent carcinogens such as *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (MNNG) (14). In our present studies, we find that daunorubicin and adriamycin transform the same cells with a frequency equivalent to that of the carcinogenic standard, MNNG. The high potency of the anthracyclines is notable, because they induce transformation in cells that have been exposed for 24 hours to concentrations as low as 0.001 μg/ml. Under similar test conditions, MNNG is at least 100-fold less potent. The transformed colonies induced by the anthracyclines are indistinguishable from those caused by MNNG. Four have been cloned and they grow on transfer-like malignant cell lines that are without contact inhibition and that form multicellular layers in criss-cross patterns. The cloned lines are currently being injected into syngeneic mice to determine their tumorigenicity in vivo.[7]

[7]We wish to acknowledge that we were encouraged to investigate the transforming activity of the anthracyclines from discussions with Dr. A. Di Marco and Dr. A. Necco, Istituto Nazionale dei Tumori, Milan, Italy, who had similar studies under way.

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Clinical Pharmacology of Adriamycin (NSC-123127)[1]

Robert S. Benjamin[2,3]

SUMMARY

In clinical pharmacology studies using adriamycin, initial plasma levels declined rapidly after intravenous administration suggesting rapid uptake into tissues. In our own studies, plasma levels decreased at an intermediate rate until 12 hours after drug administration at which time the drug disappeared with a half-life of approximately 1 day, reflecting the tissue half-life. There was extensive metabolism to adriamycinol and several aglycone metabolites. In our study, where urinary excretion was reproducible, such excretion rarely accounted for more than 10% of the administered dose in 5 days. The drug was not detected in the cerebrospinal fluid, and in one patient with an indwelling T-tube, biliary excretion of fluorescent material accounted for 40% of the administered dose, explaining the elevated and prolonged levels of adriamycin and its metabolites seen in patients with liver disease.

[Cancer Chemother Rep Part 3, vol 6:183-185, 1975]

Studies of the clinical pharmacology of adriamycin were initiated in conjunction with the drug's phase I study in Milan by Di Fronzo et al (1) using tritiated adriamycin. The radioactive drug was obtained by the addition of tritiated water to the culture medium of the *Streptomyces peucetius* var. *caesius* which produced the drug. In those studies, levels of radioactivity were measured in plasma, whole blood, red and white blood cells, urine, stool, and cerebrospinal fluid (CSF). The disappearance of radioactivity from plasma and blood was similar, with an initial rapid fall during the first 2 hours and essentially no further decline in the levels thereafter. Urinary excretion followed a similar pattern, with the majority of the label excreted during the first day followed by slow excretion with a half-life of about 2 days and a total of approximately 25% appearing in the urine over a 1-week period. In the feces, approximately 40% of the administered dose could be recovered over a similar period of time in most of the patients. In two patients, however, only 15% of the dose was recovered, due to either hepatic malfunction or incomplete stool collection. No radioactivity could be detected in the CSF. Following in vitro incubation, the drug was concentrated 500 times in white blood cells but not in red blood cells. Although these studies yielded some important information (especially regarding lack of penetration into the CSF, concentration in white blood cells, and the possible significant contribution of biliary excretion), the interpretation of their details with regard to plasma drug levels and urinary excretion are complicated by difficulty in interpreting the significance of the levels of tritium measured. It has been previously shown by Bachur et al (2) and Alberts et al (3) that for daunorubicin[4] the levels of radioactivity remained high while the levels of fluorescent materials more closely related to the native drug fell progressively. Indeed, the long plasma half-life in Di Fronzo's study is quite similar to that of tritiated water, which might result from tritium exchange in the body. These factors could also explain the relatively high urinary excretion (vide infra) since tritiated water, daunosamine, and other end products of drug metabolism would likely be excreted in the urine. On the other hand, such compounds would be unlikely to undergo biliary excretion, suggesting that the recovery of 40% of the radioactive label in the feces represented either native drug or early metabolites.

Rosso et al (4) measured adriamycin levels in plasma and urine of patients receiving low doses of adriamycin. They used a fluorometric assay after *n*-butanol extraction of salt-saturated samples. They found extremely variable plasma levels and urinary excretion. No data were supplied regarding the state of hepatic function in their patients. These results are difficult to explain in view of our own findings (vide infra), but may be due to *(a)* the inefficiency of their extraction techniques (especially for plasma), *(b)* the low dosage of drug employed, and *(c)* for urine, the magnification of any error introduced in the analysis by the calculation of 24-hour urinary drug-excretion based on analysis of a 1-ml sample. In addition, it is also possible that several of their patients had significant liver disease, which may have contributed to the variability seen in their pharmacokinetic determinations. No metabolites of adriamycin were reported by either Di Fronzo et al (1) or Rosso et al (4).

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In our initial studies on adriamycin (5), we utilized a modification of the technique previously used in the study of daunorubicin (3,6). Plasma was extracted with an acid-alcohol solution and analyzed fluorometrically. Plasma metabolites were determined by thin-layer chromatography after hydrolysis of the acid-alcohol extracts and further extraction of the resulting aglycones into ethyl acetate. Urine was extracted utilizing a nondestructive technique with *n*-butanol after salt saturation. This technique was similar to that used earlier by Rosso et al (4) except that 25-ml samples were analyzed in our study as opposed to 1-ml samples in the previous study. Our results differed significantly from those previously reported. In the plasma we noted an initial rapid decline in the plasma levels as had been seen by the other investigators; however, thereafter we noted a definite slow fall in plasma levels with a half-life of approximately 1 day. In addition, our chromatographic analysis showed small concentrations of a number of fluorescent metabolites of adriamycin. Although the individual metabolites could not be quantified in those studies, the pooled metabolites accounted for 80%-90% of the total fluorescence seen. We could not prove that the materials in the plasma were metabolites; however, supportive evidence for this concept was found in the pharmacokinetic behavior of these materials which had a half-life longer than that of adriamycin. For both adriamycin and the metabolites, the plasma disappearance followed first-order kinetics well after division into two phases.

In the urine, we noted that excretion was not variable in 23 patients (5), in distinct contrast to the report of Rosso et al (4). In our patients the cumulative urinary excretion over a 5-day period was about 6% of the administered dose. This figure is significantly lower than that reported by either Rosso et al (4) or Di Fronzo et al (1), perhaps for the reasons mentioned above. Even assuming an inefficiency of our extraction technique, it is of note that the cumulative excretion of daunorubicin over a similar period of time by the same technique was about 25% of the administered dose (6). The *n*-butanol extraction technique used in the analysis of urine samples was nondestructive and allowed for substantial concentration of the specimens. On analysis by thin-layer chromatography, it was apparent that one major metabolite as well as several minor metabolites were present. The metabolites were quantified by thin-layer chromatography in eight patients, and the most prominent urinary species was native adriamycin, followed by a metabolite which we have subsequently identified as adriamycinol (7,8).

In five patients with severe liver disease, variable plasma levels in terms of pharmacokinetics were observed. In all patients, the levels were elevated and the rate of elimination of fluorescence from plasma was prolonged (9). Examination of the records of these patients revealed fatal or life-threatening toxic effects. On that basis, we have subsequently recommended that the dosage of adriamycin be decreased in patients with liver disease.

Because of our concern that the plasma metabolites which we had initially described might be artifacts, we devised a new, nondestructive technique for extraction and chromatographic separation of adriamycin and its metabolites from plasma in native form (8). In that study, we were able to confirm our original observations about the metabolism of adriamycin. In addition, we were able to separate adriamycinol and two aglycone metabolites which were different from adriamycin aglycone as the most prominent species in human plasma after adriamycin. Although the plasma pharmacokinetics of total drug fluorescence followed a biphasic pattern as we had previously described, detailed analysis of the pattern of disappearance for chromatographically isolated adriamycin revealed three phases: the first with a half-life of approximately ½ hour, the second with a half-life of approximately 8 hours, and the third beginning 12 hours after drug administration with a half-life of approximately 28 hours. Careful analysis of our initial data on adriamycin confirmed that they also fit this pattern better than they fit our previously described biphasic pattern. Some of the other materials exhibited different pharmacokinetic patterns, and in the case of one aglycone metabolite, variability was noted within our patients, with one group exhibiting an initial rapid rise and the other group having basically stable levels.

We have also confirmed our original observations of elevated and prolonged levels of adriamycin and metabolites in patients with liver disease (10). We have noted, however, that only patients with elevated serum bilirubin have uniformly elevated and prolonged levels of adriamycin and its metabolites. In patients with other abnormalities of liver function, the results are extremely variable and cannot be well predicted. In patients who did have trouble eliminating adriamycin metabolites however, we noted that there was proportionately more increase in the levels of metabolites than in those of native adriamycin. Since the patients with such elevated levels have increased toxic reactions, these findings suggest that toxicity and possibly drug efficacy may be related to the metabolites as well as to the native drug (10).

In two patients with severely abnormal renal function, we have been unable to demonstrate abnormalities in adriamycin pharmacokinetics or toxic reactions. We have been unable to isolate adriamycin or any of its fluorescent metabolites from CSF, even in patients receiving the drug while they had evidence of cerebral metastasis with positive brain scans or meningeal leukemia (5). In one patient with an indwelling biliary T-tube, we measured adriamycin levels in the bile (11). The patient had normal hepatic and renal function at the time of study, and his plasma pharmacokinetics of adriamycin were normal. We recovered 40% of the administered fluorescent material over a 1-week period and approximately ten chromatographically separable fluores-

cent species were detectable; however, the most prominent material was adriamycin and the next most prominent was adriamycinol. Several conjugated compounds as well as several aglycones were present in smaller amounts.

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Adriamycin (NSC-123127) by Arterial Infusion[1,2]

Charles M. Haskell, Frederick R. Eilber, and Donald L. Morton[3,4]

SUMMARY

Arterial infusion is an experimental route for administering adriamycin. The systemic toxicity of intra-arterial adriamycin appears to be equivalent to that seen when the drug is given intravenously. Local complications, however, are more common when the drug is administered intra-arterially. At the present time there is no established indication for the use of intra-arterial adriamycin as a single modality of therapy for any human neoplasm. Its use as part of a program of multimodality therapy, primarily in patients with sarcomas, remains an active area of clinical investigation.

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With rare exceptions, the administration of anticancer drugs by an arterial route is experimental therapy. This specialized route of administration is of interest because of the potential advantage of delivering active drugs in higher concentrations to tumors without an increase in systemic toxicity (1). Drug administration by an arterial route is used primarily in the treatment of neoplasms of the liver; cancer of the head and neck has also been treated in this way, but with less success. In view of the substantial antitumor effect of adriamycin when given intravenously (iv) to patients with many kinds of tumors (2), and its known avidity for nuclear DNA of tumor cells (3), it would appear that adriamycin is a reasonable candidate for experimental use employing the intra-arterial route. Data on 42 patients treated by this route of administration are available for review. Thirty-two of these patients have been treated at the National Cancer Institute in Milan, Italy (4), and the remainder have been treated at the University of California at Los Angeles (UCLA) (5). This report summarizes these findings, and updates the status of the previously described patients treated at UCLA.

METHOD OF INFUSION

Adriamycin is freely soluble in 0.9% NaCl solution or in 5% dextrose in water. The Milan group has used heparin as part of the infusion solution, but the UCLA group has not due to problems of precipitation. Both groups have primarily utilized the drug mixed in 500-1000 ml of 5% dextrose in water for administration via a plastic infusion bag to which external pressure was applied with a blood pressure cuff. At UCLA the maximum duration of infusion was 72 hours, while the Milan group has administered the drug from 2 to 20 days. The median duration of infusion for the Milan group has been 9 days. The dose of adriamycin utilized by UCLA has been in the range of 45-107 mg/m^2 of body surface area, with the most common dose schedule being 25 mg/m^2/day for a total of 75 mg/m^2. The usual dose used in Milan has been 0.3 mg/kg/day, although in some cases 0.2 mg/kg/day was used or 0.4 mg/kg was used on alternate days.

Placement of the intra-arterial infusion catheters, by both UCLA and the Milan group, was accomplished either percutaneously or surgically by direct vision. Percutaneous placement by the Seldinger technique was possible in most cases for drug administration to the extremities or abdomen, whereas surgical placement was necessary in most cases for head and neck cancer.

ANTITUMOR RESPONSE

Four of seven patients, previously reported by UCLA, had prompt objective responses. These responses have been previously published and will not be described in detail (5), but a brief comment will be added to bring the long-term course of these patients up to date. Patient No. 1, with recurrent osteogenic sarcoma of the mandible, had a 33% decrease in the size of his tumor (product of greater and lesser diameters) after an adriamycin infusion of a total dose of 95 mg/m^2. One half of this dose was administered into the left superficial temporal artery and the other half into the right superficial temporal artery. Further tumor shrinkage was observed with the addition of preoperative radiation therapy and he subsequently underwent a wide local excision of his lesion. His mandible was surgically reconstructed, and

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[2]Adriamycin: CAS reg. No. 23214-92-8. Supplied by Drug Research and Development, Division of Cancer Treatment, NCI.

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he remains free of recurrent disease 27 months after the intra-arterial administration of adriamycin.

Patient No. 2, who was the initial patient treated at UCLA by arterial infusion, is also the only patient in the series who received the drug directly into a tumor vessel. Prior to referral to UCLA she had been found to have an inoperable leiomyosarcoma of the retroperitoneum. Upon evaluation at UCLA she was found to have an obstructed inferior vena cava, and an arteriogram showed a highly vascular tumor that was receiving 60%-70% of its blood supply from what appeared to be the right ovarian artery. She received a 3-day infusion of adriamycin into the tumor vessel at a dose level of 72 mg/m^2. By 6 days the mass had decreased to one fourth its former size and she was given preoperative radiation therapy. An abdominal celiotomy was performed on January 3, 1973, at which time a small amount of residual tumor was removed. She received additional radiation therapy which was directed to the clips placed at the site of residual tumor, and she was subsequently given immunotherapy with bacillus Calmette-Guérin and a tumor-cell vaccine. She has remained free of recurrent sarcoma for more than 2 years.

The other two patients in the original UCLA series who responded were patient No. 3, with aggressive fibromatosis of the right leg, and patient No. 4, with stage III neuroblastoma. In both patients the therapeutic benefit of adriamycin was short-lived, and therapy was subsequently changed.

The Milan group has reported the treatment of 32 patients with various tumors (4). Twenty-six of these patients were evaluable for antitumor response, including one patient with cancer of the breast, nine with cancer of the head and neck, one with reticulum cell sarcoma of the limb, one with ovarian carcinoma, one with cervical carcinoma, nine with liver and/or abdominal tumors, two with melanoma of the limb, one with bladder cancer, and one with an axillary sarcoma. Sixty-five percent of these patients showed some form of regression. Ten patients showed less than a 50% decrease in tumor size, while seven patients showed tumor regressions greater than 50%. Regressions greater than 50% were seen in one patient with cancer of the breast, in four with cancer of the head and neck, in one with ovarian carcinoma, and in one with reticulum cell sarcoma of the liver in whom a prompt and impressive regression of hepatomegaly was observed. The only patient with a sarcoma had a regression which was less than 50% in extent, and this patient subsequently received radiation therapy.

TOXICITY

The toxic manifestations of intra-arterial adriamycin, as employed at UCLA, appear to be somewhat fewer than those seen in the program employed by the group in Milan. At UCLA, stomatitis developed in four of ten patients, but only one of these patients had severe stomatitis. Alopecia occurred in all but two patients, and infection occurred in three patients. Serious infections occurred in only two patients, and were associated with marked leukopenia with white blood cell counts (wbc) of 1100 and 2300 cells/mm^3, respectively. In neither patient was the infection fatal, since both patients responded to antibiotic therapy. The doses of adriamycin associated with this severe degree of leukopenia included 91 and 100 mg/m^2, respectively. The lowest wbc observed in patients treated at UCLA at dose levels between 60 and 80 mg/m^2 was 2300 cells/mm^3 in one patient, and this patient had a minor infection at the catheter site which responded to local antibiotic therapy. In addition, most of the patients treated at UCLA noted mild anorexia and nausea. Also, a clotted catheter in one patient had to be replaced; there were two episodes of trismus in one patient related to high rates of drug administration through the superficial temporal arteries; and there was one episode of facial edema followed by skin necrosis in a patient who had received extensive previous radiation therapy. This problem ultimately healed with local care, and we believe that it relates directly to the previous administration of high-dose radiation therapy to that region.

The toxicity reported by the Milan group has included two deaths. One death was attributed to sepsis related to agranulocytosis, and the second patient died of what was considered most likely to be cardiac toxicity. Of the 32 patients in the series, 17 had stomatitis, 17 had alopecia, 12 had leukopenia (wbc less than 3000 cells/mm^3), six had thrombocytopenia (platelet count less than 100,000 platelets/mm^3), 11 had anemia (hemoglobin less than 9 g/100 ml), seven had fever during the period of drug administration, and five had cardiac toxicity. Four of the five patients with cardiac toxicity manifested this effect as a change in ECGs which were characterized by depression of the ST segment and flattening or inversion of the T waves. The patient who died of presumed cardiac toxicity was a 52-year-old man with carcinoma of the liver. The ECG was normal prior to the administration of adriamycin, and he subsequently received a total dose of 6.3 mg/kg of adriamycin. The ECG became abnormal and he also had associated anemia. Death occurred suddenly on a presumably cardiac basis, but there is no additional information in the report to substantiate this opinion.

DISCUSSION

The intra-arterial administration of adriamycin remains an experimental approach to therapy. The results described in this paper from Milan and UCLA underline this conclusion. The approach to therapy in these two centers is quite different, in that the Milan group has used the arterial route of administration with adriamycin in an attempt to induce remissions which may be subsequently maintained by other forms of nonsurgical therapy. They

have concluded from their experience that the antitumor effect of adriamycin, when administered via this route to the patients under their care, was essentially equivalent to that which can be achieved with iv administration of the drug. The higher rate of complications, both systemically and locally from the impact of the catheter on the vascular system, has led them to abandon the use of this approach to adriamycin administration. The UCLA experience would certainly support this recommendation for the specific setting of adriamycin given as the sole form of therapy to patients with metastatic neoplasms. A possible exception to this overall conclusion, however, might be the intra-arterial administration of adriamycin to patients with hepatomas. Preliminary data suggest that adriamycin administered iv may be useful in treating this disease (6). Since adriamycin appears to be metabolized by the liver (7), it is possible that hepatic artery infusion of adriamycin may allow a more aggressive adriamycin treatment of hepatomas without a concomitant increase in systemic toxicity.

In contrast, the intra-arterial administration of adriamycin may be useful as part of a program of multimodality therapy, particularly for patients with well-defined tumor vessels. Indeed, the initial patient treated at UCLA by this route was a patient with an inoperable leiomyosarcoma which was receiving its arterial blood supply from a specific tumor blood vessel. Adriamycin was part of a program of therapy which has given this patient more than 2 years of disease-free survival. Because of this early success, the use of intra-arterial adriamycin as part of a program of multimodality therapy continues to be the subject of active research interest at UCLA.

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A Practical Approach to Adriamycin (NSC-123127) Toxicology[1]

Robert S. Benjamin[2,3]

SUMMARY

The significant complications of adriamycin therapy, discovered during the initial therapeutic trials, are myelosuppression, mucositis, cardiomyopathy, and local tissue necrosis. Risk factors for myelosuppression include infiltration of tumor into bone marrow, prior irradiation therapy (and chemotherapy only when associated with cumulative myelosuppression), and liver disease. The dangers of myelosuppression are minimized by scheduling intermittent doses, repeated no more frequently than at 3-week intervals. Guidelines for dosage reduction in the presence of liver disease are presented. Mucositis is an unusual side effect of intermittent single-dose administration. Cardiac toxicity is not usually seen if the cumulative dose of adriamycin is less than 550 mg/m² in most patients and less than 450 mg/m² in patients with risk factors of mediastinal irradiation and/or simultaneous cyclophosphamide. Local tissue necrosis is minimized by intermittent administration of single doses since fewer injections are given, and in addition, by meticulous care in drug administration. Minor toxic effects of adriamycin are described as well. Some of these can also be ameliorated or eliminated by care in drug administration.

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The predominant toxic manifestations of adriamycin therapy were well described following its initial phase I evaluation by Bonadonna et al (1,2). The familiar tetrad of nausea and vomiting, alopecia, stomatitis, and myelosuppression is often associated with cancer chemotherapy with a variety of agents. In addition, cardiac toxicity, previously described with daunorubicin[4] (3,4), was observed. The toxic manifestations of adriamycin seen during its initial clinical trial at the National Cancer Institute (Baltimore Cancer Research Center) (5) are shown in table 1. The various dosage schedules used by different investigators will be discussed with regard to the individual toxic manifestations.

The acute dose-limiting toxicity of adriamycin is myelosuppression. With intermittent dose scheduling the myelosuppression, although it may be severe, is rapidly reversible and platelet sparing. The nadir of leukopenia occurs 12 days after drug administration and recovery is complete by Day 19. In order to allow for recovery from bone-marrow toxicity induced by the previous dose of adriamycin, a 3-week interval is appropriate. Although myelosuppression is often severe, the very short duration of the myelosuppression makes aggressive therapy quite safe

TABLE 1.—Adriamycin toxicity in 96 patients

Effects	% of patients with effects
Serious	
Hematologic	83
Leukopenia (<3500)*	83
Severe (<1000)*	24
Related to death	6
Thrombocytopenia (<100,000)*	38
Cardiac	
Congestive heart failure	2
Fatal	2
Mucositis	9
Local tissue necrosis	0
Minor	
Alopecia	100
Nausea and vomiting	46
Facial flush	9
Fever and chills	5
Hyperpigmentation	ca 10
Rash	3
Urticaria	2
Maculopapular	1
Drowsiness	ca 40

*Cells/mm³.

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[4]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

with regard to infectious complications. In addition, there is relative platelet sparing in the absence of the risk factors we have previously described: infiltration of tumor into bone marrow, radiation therapy, and liver disease (5).

In our experience, patients with prior chemotherapy, usually with multiple single agents, did not appear to have an increased risk of bone-marrow toxicity. Of course, in patients who have received prior chemotherapy which has a cumulative myelosuppressive effect, an increased risk with any subsequent therapy is to be expected. What was important, however, was that significantly increased myelosuppression, especially with regard to thrombocytopenia, was seen in patients with prior radiation therapy, even when it was as low as 3000 rads to the mediastinum. The important point about bone-marrow infiltration and radiation therapy as risk factors, is that they decrease the therapeutic index of adriamycin chemotherapy. In some situations, decreasing the dose of adriamycin to make chemotherapy possible results in ineffective chemotherapy. For that reason particular attention to supportive care is required for patients in this category.

The problem with liver disease is quite different. Since liver disease contributes to adriamycin toxicity by increasing the effective product of concentration and time (C × T) of the administered adriamycin, decreasing the dose of adriamycin should result in values of C × T similar to those seen with higher doses in patients with normal liver function. The practical problem with adriamycin administration in patients with liver disease is the variability of adriamycin elimination in these patients. We have attempted to relate several measurements of liver function with increased plasma levels of adriamycin. However, only an elevated serum bilirubin level has been uniformly associated with abnormal adriamycin pharmacokinetics. In patients with normal serum bilirubin, we initially attempted to use the sulfobromophthalein (BSP) clearance to determine adriamycin dosage (5). In most cases, however, even with markedly elevated BSP retention, adriamycin was cleared normally. In contrast, in some patients, only mild hepatomegaly and abnormalities of liver function were associated with markedly increased adriamycin levels. Our current recommendations for adriamycin dose reduction in the presence of liver disease are shown in table 2. When the bilirubin is elevated the guidelines given have resulted in uniformly acceptable toxicity and therapeutic efficacy. In patients with normal serum bilirubin, the risk of an adriamycin overdose from full-dose therapy must be balanced against the need for an initial antitumor response. Whenever the need for rapid antitumor effect is overwhelming, full-dose adriamycin should be given to patients with normal bilirubin. The guidelines shown here are recommended for patients in whom a little more leeway exists. In all cases, the decision to choose a higher or lower dose level for adriamycin should be modified based on the other risk factors present, and subsequent doses should be escalated until the nadir granulocyte count approaches 1000 cells/mm^3 or until there is morbidity.

Initial phase I clinical trials of adriamycin by Bonadonna et al utilized a variety of dose schedules (1,2). The majority of these involved intermittent administration of small doses of adriamycin, daily or every other day for a few doses, followed by a short and variable rest period, and then either repetition of the initial loading dose or weekly or biweekly maintenance therapy. In the early patients stomatitis was frequently the first sign of toxicity, with the potential of irreversible myelosuppression if therapy was not interrupted at the onset of stomatitis (2). In addition, the high frequency of stomatitis and other evidence of mucositis made adriamycin therapy quite unpleasant to the patients.

The intermittent loading-dose schedule, introduced by Middleman et al (6) and Wang et al (7), was subsequently adopted by Bonadonna et al (8). The predominant advantage of that dose schedule was that myelosuppression became predictable and reversible. In addition, when lower doses were used (20-25 mg/m^2 daily × 3), the incidence of stomatitis fell significantly to 39%. This schedule has been used extensively in the studies of the Acute Leukemia Group B (ALGB) under Cortes et al (9-11) and in pediatric studies by Tan et al (12). At the higher doses used by the ALGB (30-35 mg/m^2 daily × 3), however, the incidence of mucositis increased again to 88%.

TABLE 2.—Adriamycin administration in liver disease

Patients with—	If patients have—		Then adriamycin dose should be—*
	Serum bilirubin	Serum transaminase	
Elevated serum bilirubin	1.2-3 mg/100 ml		½ normal
	>3 mg/100 ml		¼ normal
Normal serum bilirubin		2-3 × normal	¾ normal
		>3 × normal	½ normal

*Modify dose based on other risk factors; escalate subsequent doses.

Single-dose administration of adriamycin was used initially by Kenis et al (13,14) in Belgium, and shortly thereafter in the United States by our group in Baltimore (5,15) and Frei et al (16) at the M. D. Anderson Hospital at Houston. The studies in Baltimore and those in Belgium were based on the pharmacologic observations of the long plasma half-life of adriamycin (5,15,17,18). Kenis was the first to report the striking reduction in stomatitis when intermittent single doses of adriamycin were employed (13). These findings were confirmed in our initial studies (5, 15) and in those from the M. D. Anderson Hospital utilizing adriamycin both as a single agent (16) and in combination with 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide[5] (19) as well as a number of other agents. The overall relation of stomatitis to dosage schedule is shown in table 3. These differences are all statistically significant (5). Of interest are the risk factors of liver disease without appropriate dosage reduction and prior radiation therapy. Mucositis can involve any area of the gastrointestinal tract. Esophagitis from adriamycin has, in our experience, been seen only in patients who have undergone prior radiation therapy to that area. The factor of liver disease has been discussed in the section on myelosuppression.

The markedly decreased incidence of mucositis in patients receiving single-dose therapy as opposed to those receiving the drug in 3-day courses is the reason why we and others prefer to use adriamycin by the intermittent single-dose schedule. The largest studies of this type, which have been carried out by O'Bryan et al (20,21) in the Southwest Oncology Group based on the pilot study at the M. D. Anderson Hospital (16), confirm the low incidence of mucositis and the significant clinical activity utilizing this schedule. In addition, there is a suggestion of a dose-response relationship, at least for sarcomas, lymphomas, and bladder carcinoma (21). Other dosage schedules including weekly therapy without a loading dose, 23-hour infusions, divided doses, and massive doses have been utilized. Data are preliminary, but they do not show a significant advantage in increased efficacy or decreased toxicity, except possibly for the infusion therapy where the risk of extravasation and severe local necrosis far outweighs any possible advantage.

Data on cardiac toxicity are presented in detail in another paper in this symposium (22). The risk factors include mediastinal irradiation, simultaneous cyclophosphamide,[6] increased dose of adriamycin per unit time, and possibly pre-existing conditions such as hypertension which may increase cardiac work. Cardiac toxicity is rarely noted below a cumulative dose of 550 mg/m^2. Using this guideline as an endpoint for adriamycin therapy in patients without these risk factors, the risk of cardiac toxicity will be very low. A further reduction in the dose limitation to 450 mg/m^2 is recommended in the presence of these risk factors.

TABLE 3.—Relation of stomatitis to dosage schedule

Schedule	Incidence of stomatitis (%)
Prolonged low doses	65
Intermittent loading dose (20-25 mg/m^2 qd × 3)	39
Intermittent single dose (60-75 mg/m^2)	7

Local tissue necrosis from adriamycin extravasation can be a tremendously debilitating complication, and if infection occurs, this effect is potentially lethal during further myelosuppressive chemotherapy. This complication is proportional to the number of injections given rather than to the total dose, and is therefore another reason to support therapy with the fewest number of injections, namely the intermittent administration of single doses. In addition, careful attention to the technique of adriamycin administration will further reduce the incidence of this complication. Adriamycin should always be administered through an infusion set rather than a straight needle. Unless the vein is large, the drug should be administered by direct intravenous infusion of a highly diluted solution or by injection into the side arm of an intravenous infusion of saline or 5% dextrose and water. There should be adequate blood return, and infusions should be avoided around joints where the needle has a greater chance of dislodgement during drug administration. Adriamycin usually hurts when it extravasates; therefore, if the patient complains of stinging, burning, or hurting during adriamycin administration, it should be terminated immediately even if blood return can be obtained. Of course, this type of drug should not be administered in a leg vein unless absolutely necessary, since amputation may be the only way of preventing infection in an area of extravasation-necrosis. For similar reasons, the nondominant arm is preferable if the choice exists. Our nurses have noted that the immediate application of ice packs to an area of adriamycin extravasation, when maintained for a full 24 hours, often decreases the severity of the necrotic reaction.

Phlebosclerosis frequently occurs when a high concentration of adriamycin is administered in small veins. For this reason, it is advisable to alternate veins whenever possible, and to administer the drug directly only when the veins are large.

Flushing is an unusual side effect of adriamycin which is almost always due to overly rapid drug administration. If the drug is given over a period of at least 2 minutes, flushing can be essentially eliminated as a complication.

The cutaneous manifestations of adriamycin toxicity include reactivation or radiation dermatitis

[5]5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide: NSC-45388; CAS reg. No. 4342-03-4; DTIC.

[6]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,-3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

(first described by Gottlieb et al [23] and similar to that frequently seen with actinomycin D [NSC-3053]), hyperpigmentation (24), nonspecific dermatitis, and urticaria (25). The urticaria may not be allergic in etiology but due to local toxicity and histamine release. It is rarely associated with systemic anaphylaxis and is not an indication to discontinue therapy.

Nausea and vomiting from adriamycin occurs in less than one half of the patients, is considerably less frequent and severe than with many other antitumor agents, and is often relieved by antiemetics.

Many patients complain of drowsiness during the 24 hours following adriamycin administration. Although we have not specifically evaluated the incidence of this reaction, it probably occurs in one third to one half of all patients, most often in those without predominant gastrointestinal toxicity. The mechanism is unclear, since adriamycin does not appear to get into the central nervous system (5,18).

Fever and chills are seen occasionally, but less commonly than with daunorubicin (26).

In conclusion, certain guidelines have been developed by careful attention to the details of adriamycin toxicology which should decrease the incidence or eliminate many of the serious and unpleasant side effects associated with the use of this important new antitumor antibiotic.

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Adriamycin (NSC-123127) Cardiomyopathy—An Overview With Determination of Risk Factors[1,2]

Robert A. Minow, Robert S. Benjamin, and Jeffrey A. Gottlieb[3,4]

SUMMARY

The clinical and pathologic features of adriamycin cardiomyopathy are reviewed. Two types of cardiac effects are attributed to adriamycin: (1) various nonspecific electrocardiogram abnormalities, and (2) dose-related congestive heart failure. Attempts are being made to detect adriamycin-induced cardiotoxicity before clinically significant damage has occurred. The systolic time interval, echocardiograms, and electrocardiogram limb lead QRS voltages are discussed. In addition, several factors which increase the risk of cardiac toxicity developing have been identified. Cardiomyopathy developed at lower cumulative doses of adriamycin in patients who had had prior radiation therapy in which any portion of the heart was included in the treatment field or in patients who had received cyclophosphamide concurrently with adriamycin. We are presently limiting these patients to a cumulative dose of adriamycin of 450 mg/m^2.

[Cancer Chemother Rep Part 3, vol 6:195-201, 1975]

The application of adriamycin has been limited to induction or consolidation therapy rather than long-term maintenance largely because of severe dose-dependent cardiomyopathy.

The symptoms, physical examinations, and laboratory findings of adriamycin cardiomyopathy are those expected for congestive heart failure (CHF). The most striking characteristic is the insidious onset of symptoms followed shortly by rapidly progressive CHF. Extreme care must be taken to avoid giving even one additional dose of adriamycin to an already severely damaged heart. The first symptom may be as nonspecific as a dry nonproductive cough suggesting the diagnosis of bronchitis rather than adriamycin cardiotoxicity. Any new symptom or physical finding should be thoroughly investigated and a cardiac etiology ruled out before additional adriamycin is administered. Chest roentgenograms should be taken frequently to detect cardiomegaly or pleural effusions. Abnormalities of the cardiac enzymes have not been noted on serial determinations (1).

Various other abnormalities are associated with adriamycin cardiomyopathy. Minow et al (2) have documented the decreased QRS voltage on the electrocardiogram (ECG), Rinehart et al (3) have shown an increase in the systolic time interval (STI), and Jones et al (4) have recorded a decrease in the ejection fraction as measured by echocardiography. A decrease in fractional shortening may be an even more sensitive echocardiogram measurement (5). The therapy is the same as for other cardiomyopathies, and absolute bed rest, digitalis, and diuretics are important but may be ineffective.

Pathologic examination in cases of adriamycin cardiomyopathy (1) reveals the heart to be enlarged, pale, and flabby. The ventricles are dilated and they may be mildly hypertrophied. Mural thrombi associated with multiple pulmonary or systemic emboli are occasionally found. The coronary arteries and cardiac valves are normal.

Light microscopic examination of the myocardium reveals a decreased number of myocardial cells with degeneration of the remaining cells. There is interstitial edema, and if the patient survives the acute episode, interstitial fibrosis is seen. Signs of inflammation, either acute or chronic, are conspicuously absent.

Electron microscopy shows a continuation of these changes on the cellular level. Cellular loss and degeneration are again seen. The Z-line substance is distorted with some myofibrils showing only Z-line irregularity while in others there is marked disruption of the Z-line. The mitochondria are swollen, their cristae are disrupted and they contain dense inclusion bodies. Glycogen granules are not present.

Cortes et al (6) reported on the autopsies of 46 patients who received various doses of adriamycin without the occurrence of CHF. Twelve cases (26%) showed pathologic evidence of cardiotoxicity. These changes were less severe than those seen in autopsies of patients who did have CHF.

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[2]Adriamycin: CAS reg. No. 23214-92-8.

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Case reports documenting the clinical and pathologic findings of adriamycin cardiomyopathy have been previously reported (1).

DISCUSSION

Two cardiac effects are attributed to adriamycin: *(a)* various changes in the ECG, and *(b)* CHF.

Changes in ECG occurred in 160 of 1051 patients (15.2%) treated with adriamycin (6-9) (table 1). The most frequent ECG changes (1) were: *(a)* abnormalities of cardiac rhythm, including supraventricular tachyarrhythmias and premature ventricular, and supraventricular contractions; *(b)* abnormalities of conduction such as left axis deviation and decreased QRS voltage; and *(c)* a variety of nonspecific ST-T wave abnormalities. All these abnormalities are nonspecific, occur at all dose levels irrespective of schedule, and except for the decreased QRS voltage resolve 1-2 months after discontinuing adriamycin therapy. If the initial ECG is abnormal new abnormalities are more likely to develop than if the initial ECG is normal (6,8,10) (table 2). However, the development of ECG abnormalities does not predict the development of CHF and is not an indication to discontinue adriamycin.

Cardiomyopathy developed in 1.8% (31 of 1758) of the patients who received adriamycin (7-9,11,12) (table 3) and was irreversible in 59%, with death usually occurring within 3 weeks. The total dose of adriamycin correlated well with the development of cardiomyopathy (6,13,14) (table 4). Cardiomyopathy developed in only 0.3% of all patients who received adriamycin doses of 500 mg/m^2 or less. The incidence increased to 11% when doses of 501-600 mg/m^2 were given and to 31% when doses greater than 600 mg/m^2 were administered. Observing the patients in 50-mg/m^2 dose intervals (table 5) (eliminating only Bonadonna's patients who were separated into 100-mg/m^2 intervals), none of the 738 patients shown had cardiomyopathy at doses below 450 mg/m^2. Even though several patients have since developed cardiomyopathy at doses below 450 mg/m^2, the incidence is still very low. None of the 26 patients who received doses of 451-500 mg/m^2 and 9% of the patients who received doses of 501-550 mg/m^2 had cardiomyopathy. The marked increase occurs in the interval of 551-600 mg/m^2 where 20% of the patients had cardiomyopathy. Thirty-five percent of the patients who received doses greater than 550 mg/m^2 had cardiomyopathy. This is why we are now limiting all patients, with several exceptions to be noted below, to a total dose of 550 mg/m^2.

Kushner et al (15) have described in detail a patient in whom a cumulative dose of 600 mg/m^2 was well tolerated. He was then maintained on other drugs for 13.5 months but because of progression of disease it was necessary to risk reinstituting adriamycin. Over the next 3 months, he received four additional courses of adriamycin for a total dose of 120 mg/m^2. Two months later he had severe CHF. Benjamin et al (16) have also described an 18-year-old man who received a dose of 420 mg/m^2 of adriamycin. After a 6-month hiatus adriamycin was reinstituted. Following an additional dose of 180 mg/m^2 fatal cardiotoxicity developed. These cases illustrate that at least in 1 year the heart does not recover from the previous subclinical cardiac damage produced by an initial course of adriamycin. Therefore, once a patient has received a full course of adriamycin he probably should never receive any additional adriamycin.

TABLE 1.—Frequency of adriamycin ECG changes

Investigators	Ref No.	No. of patients treated	No. of patients with ECG changes (%)
Gottlieb et al	7	760	77 (10.1)
Bonadonna et al	8	131	54 (41.2)
Cortes et al	6	100	25 (25.0)
Tan et al	9	60	4 (6.7)
Totals		1051	160 (15.2)

TABLE 2.—Adriamycin ECG changes: effect of previous abnormalities*

	No. of patients treated	No. of patients with normal or unchanged ECGs (%)	No. of patients with new abnormalities (%)
Initial ECG			
Normal	188	141 (75)	47 (25)
Abnormal	84	48 (57)	36 (43)

*See ref Nos. 6, 8, and 10.

TABLE 3.—Incidence of adriamycin cardiomyopathy

Investigators	Ref No.	No. of patients treated	No. of patients with cardiomyopathy (%)	No. of patients who died
Gottlieb et al	7	760	12 (1.6)	9
Bonadonna et al	8	553	9 (1.6)	4
Cortes et al	12	115	7 (6.1)	3
Benjamin et al	11	96	2(2.1)	2
Tan et al	9	234	1 (0.4)	1
Totals		1758	31 (1.8)	19

TABLE 4.—Dose-related incidence of adriamycin cardiomyopathy*

Total dose (mg/m²)	No. of patients— At risk	With cardiomyopathy	Frequency of cardiomyopathy (%)
≤500	1254	4	0.3
501-600	66	7	11
> 600	61	19	31

*See ref Nos. 6, 13, and 14.

TABLE 5.—Dose-related incidence of adriamycin cardiomyopathy using 50-mg/m² dose intervals*

Total dose (mg/m²)	No. of patients— At risk	With cardiomyopathy	Frequency of cardiomyopathy (%)
≤450	738	0	0
451-500	26	0	0
501-550	32	3	9
551-600	15	3	20
> 600	37	15	41
Totals > 550	52	18	35

*See ref Nos. 6 and 13.

Other drugs may potentiate the cardiotoxic effect of adriamycin. The CHF of the patient described by Kushner et al (15) seemed to be exacerbated by actinomycin D and mithramycin.[5] At present, we have not seen any similar examples.

Because 65% of the patients would not have cardiomyopathy even if the dose of adriamycin exceeded 550 mg/m², and a few patients would have cardiomyopathy at lower cumulative doses, tests which might predict adriamycin cardiotoxicity and allow for a more accurate dose limitation are being investigated. Weissler et al (17, 18) have shown that the STI detects left ventricular dysfunction in patients with heart failure. The STI is the ratio of the pre-ejection period (PEP) to the left ventricular ejection time (LVET) as measured by simultaneous recordings of the phonocardiogram, carotid artery pressure, and ECG. In patients with heart failure there is prolongation of the PEP and shortening of the LVET while the entire period of electromechanical systole remains unchanged. As a result, the ratio of PEP/LVET, ie, the STI, increases in patients with heart failure.

Rinehart et al (3) recently recommended the use of the STI for the early detection of adriamycin cardiotoxicity. Eight of their nine patients (89%) who received doses of adriamycin greater than 300 mg/m² had a significant increase in the STI. They suggest that an increase of the STI of 0.08 correlates with significant adriamycin cardiotoxicity. If so, this would allow for a maximum dosage of adriamycin without excessive risk of life-threatening heart failure.

[5]Actinomycin D: NSC-3053.
Mithramycin: NSC-24559; CAS reg. No. 18378-89-7.

Without knowledge of the STI we gave adriamycin to 54 patients at doses of 451-550 mg/m² (13). These patients have been followed for a minimum of 6 months. Extrapolating from the data of Rinehart et al (3), we would have expected 48 of the 54 patients (89%) to have had a significant increase in the STI, but only one of these patients had clinical cardiac toxicity, an incidence of only 1.8%.

In the uniformly fatal malignant diseases in which it is used, adriamycin can result in frequent prolonged objective responses. The potential benefit of additional adriamycin must be weighed against the risk of potentially fatal cardiac damage from its continued administration. If the cumulative dose of adriamycin is kept below 550 mg/m² and clinical parameters are followed closely, the risk is quite low. Although the STI detects left ventricular dysfunction, we believe it is too sensitive an indicator. We do not feel that a significant increase of the STI *alone* is an indication to discontinue adriamycin, as many patients would be deprived of the potential benefit of further adriamycin therapy while only a few would be spared cardiac toxicity.

In addition, it was hoped that a normal STI would give early warning of impending cardiotoxicity to allow patients to exceed a total dose of 550 mg/m². As the following case report demonstrates, this may not be the case.

Case Report

A 30-year-old man underwent an exploratory laparotomy for intestinal obstruction. An undifferentiated retroperitoneal sarcoma with multiple abdominal metastases was found. Chemotherapy consisting of cyclophosphamide, vincristine, adriamycin, and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (19) was administered at 4-week intervals with resulting stabilization of his disease.[6] Monthly physical examinations, chest roentgenograms, and ECGs were normal including an examination done 4 weeks after his last dose of adriamycin. In addition, STIs performed 4 weeks after he had last received adriamycin at cumulative doses of 350, 450, and 550 mg/m² were all normal (fig 1). Therapy with adriamycin was stopped after a total dose of 550 mg/m². Despite this, severe CHF developed 3 ½ months after a normal STI. His STI became abnormal and his ECG showed a marked decrease in the QRS voltage especially in the limb leads. Fortunately, the patient responded to therapy which included bed rest, digitalis, and diuretics, but he still has severe exercise intolerance 7 months after the development of CHF. Thus, the STI can be normal when adriamycin is discontinued yet cardiotoxicity may still develop. We

[6]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,-3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).
5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide: NSC-45388; CAS reg. No. 4342-03-4.

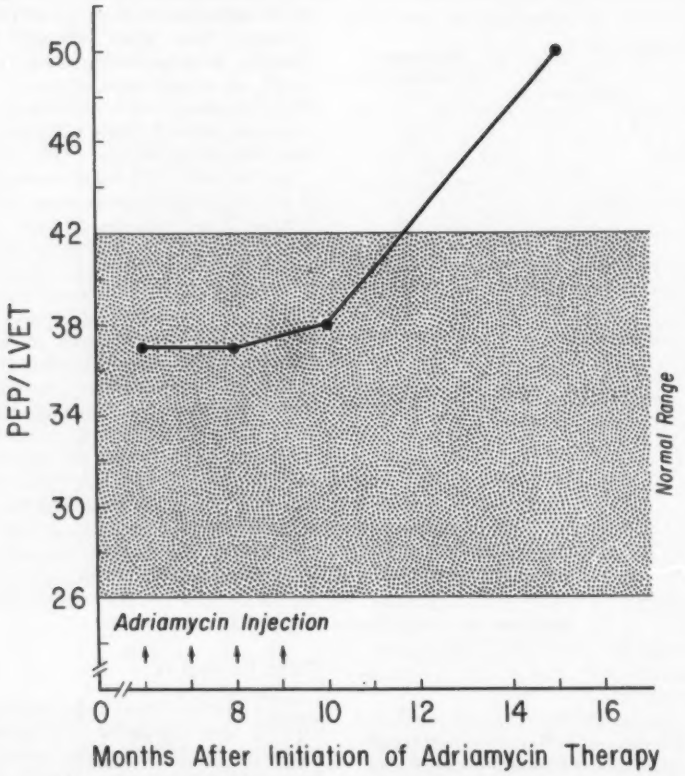

FIGURE 1.—Serial systolic time intervals in a patient with adriamycin cardiomyopathy.

believe that if we had continued to administer adriamycin in this patient until a significant increase in the STI occurred, additional cardiac damage would have developed and the patient would probably have died from cardiomyopathy. Therefore, we cannot recommend that a normal STI *alone* justifies routinely exceeding the dose limitation of 550 mg/m².

In an attempt to detect any factors which increase the risk of developing adriamycin cardiotoxicity we analyzed 17 patients who had CHF which was felt to be secondary to adriamycin (table 6). Several patients previously reported on were not included because of lack of ECG data. These patients received adriamycin at a single dose of 45-75 mg/m² every 3-4 weeks. There were six men and 11 women. Cardiomyopathy in five of the 17 patients was fatal. The median age was 51 years and the median cumulative dose of adriamycin was 510 mg/m². The median age and cumulative dose of adriamycin were similar in both the fatal and nonfatal groups. The median time from the first dose of adriamycin to the development of cardiomyopathy was 279 days; however, the pa-

TABLE 6.—Adriamycin cardiomyopathy patient characteristics*

	Total dose (mg/m²)	Age at CHF (yrs)	No. of days from— First adriamycin to CHF	No. of days from— Last adriamycin to CHF	CHF to death
Fatal (5 cases)	540 (435-825)	51 (25-59)	244 (157-282)	25 (9-50)	2 (1-10)
Nonfatal (12 cases)	510 (310-600)	52 (20-70)	330 (141-505)	54 (10-192)	
Totals	510 (310-825)	51 (20-70)	279 (141-505)	34 (9-192)	

*Values are given as medians; ranges are in parentheses.

tients that died had cardiomyopathy after a median of only 244 days while the nonfatal patients had cardiomyopathy after a median of 330 days. Since both groups received similar total doses of adriamycin, the patients that died must have received larger doses of adriamycin per course than the nonfatal patients. This was confirmed by calculating the mg/m^2/day administered to a patient using the interval from the first dose of adriamycin to the day of administration of the last dose. The patients that died received a median of 2.8 mg/m^2/day (range, 2.3-4.7 mg) while the nonfatal patients received a median of 2.35 mg/m^2/day (range, 0.9-4.1 mg). Although this is not statistically significant, the trend is suggestive. The median time from the last dose of adriamycin to the diagnosis of cardiomyopathy was 25 days in the fatal cases and 54 days in the nonfatal cases. This suggests that the patients that died had CHF more fulminantly than the nonfatal patients. This suggestion is supported by the median survival of only 2 days in the fatal cases with a range of 1-10 days.

Low QRS voltage is one of the ECG abnormalities of adriamycin. We noted that this occurred primarily in the limb leads and patients with cardiomyopathy often had this abnormality. In fact, it has been felt that this reflects the diffuse nature of the cardiac injury (1). These 17 patients had ECGs before receiving adriamycin and at the time cardiomyopathy was diagnosed.

Using only the six limb leads, measurements were made of the absolute deflection of the QRS. If there was more than one deflection in a direction only the greater deflection was used. Comparisons were made of the sums of the six limb lead measurements in the pre-adriamycin ECG and the ECG at the time cardiomyopathy was diagnosed. The median decrease in the absolute voltage of the QRS was 55% with 16 of 17 patients (94%) having a 40% or greater decrease.

Patients who had cardiomyopathy at higher total doses of adriamycin had a greater percent decrease in the QRS complexes than patients who had cardiomyopathy at lower cumulative doses. The correlation coefficient was highly significant at $P<0.02$, suggesting that the changes in the ECG voltages are continuous and might occur before overt CHF developed. We are currently evaluating a similar group of patients who did not have cardiomyopathy to determine if these QRS changes are predictive of cardiomyopathy.

Presently, changes in the total limb lead ECG voltage of 40% or greater associated with the development of *any* clinical abnormality such as a gallop rhythm, dyspnea on exertion, paroxysmal nocturnal dyspnea, or cardiac enlargement on chest roentgenogram are being used by us as an indication to withhold further adriamycin.

We had reason to suspect that two additional factors, cardiac radiation and cyclophosphamide, increase the risk of development of cardiac toxicity. First, myocardial necrosis and cardiac fibrosis have been seen after thoracic irradiation (20) and Gilladoga et al (21) noted that incidental radiation therapy to the heart increased the susceptibility of children to adriamycin cardiotoxicity. Secondly, patients receiving high-dose cyclophosphamide therapy (22), with doses in the range of 60-120 mg/kg, may on occasion have cardiac necrosis. Of even more importance, Schmidt[10] has shown that monkeys receiving cyclophosphamide concurrently with adriamycin have cardiac toxic reactions at lower cumulative adriamycin doses which ordinarily are not associated with cardiotoxicity. We then investigated these two factors in our patients.

Table 7 shows the relationship of the cumulative dose of adriamycin and concurrent chemotherapy with cyclophosphamide and/or prior radiotherapy in which any portion of the heart was included in the radiotherapy field. Of our 17 patients, 13 had cardiomyopathy at a total dose below the dose limitation of 550 mg/m^2 or less. Of these 13 patients, five had concurrent cyclophosphamide, two had prior radiotherapy alone, and two had both prior radiotherapy and concurrent cyclophosphamide. The mg/m^2/day of adriamycin for all patients was calculated. The patients who had neither concurrent cyclophosphamide nor prior radiotherapy were given a median dose of 2.82 mg/m^2/day (range, 2.36-4.12). Patients who received concurrent cyclophosphamide only were administered a median dose of 1.97 mg/m^2/day (range, 0.94-2.37) and the patients who received concurrent cyclophosphamide with or without prior radiotherapy were given a median dose of 1.97 mg/m^2/day (range, 0.94-3.83). The Wilcoxan test was

[10]Schmidt L. Personal communication.

TABLE 7.—Relationship of dose and concurrent cyclophosphamide or prior radiation therapy in adriamycin cardiomyopathy

Adriamycin cumulative dose (mg/m^2)	No. of patients with cardiomyopathy who received—				
	Neither cyclophosphamide nor prior radiation therapy	Cyclophosphamide only	Prior radiation therapy only	Cyclophosphamide and prior radiation therapy	Total
450	1*	1	—	1	3
451-500	—	2*	2	—	4
501-550	3*	2	—	1	6
Totals	4	5	2	2	13

*One patient died at this level and therapy status.

highly significant for both of these groups ($P<0.04$) when compared to the group who received adriamycin in the absence of these risk factors. Thus, we have shown that these two factors, prior radiotherapy to the heart and concurrent cyclophosphamide, potentiate the development of adriamycin cardiomyopathy.

Ten of our patients had cardiomyopathy after receiving cumulative doses between 451 and 550 mg/m^2. If we had discontinued treatment at cumulative doses of 450 mg/m^2 in all patients who had received radiation therapy to any portion of the heart and/or concurrent chemotherapy with cyclophosphamide, seven of these patients may not have had cardiomyopathy. Presently, we are limiting therapy to a cumulative dose of 450 mg/m^2 in all patients who had radiation therapy to the chest in which any portion of the heart has been included in the treatment field and all patients who are receiving cyclophosphamide concurrently with adriamycin.

Finally, we looked at prior heart disease as a possible risk factor. Only one patient had prior heart disease. This was a 48-year-old black woman who received cyclophosphamide with adriamycin. She had hypertension which could not be controlled and she had cardiomyopathy after a dose of only 310 mg/m^2. The normal heart hypertrophies in response to hypertension. Adriamycin interferes with this process, and in fact causes a loss of myocardial cells preventing this normal protective mechanism. It would seem that less cardiac damage would be required to produce CHF in this situation. Factors known to influence cardiac workload and result in cardiac hypertrophy (such as hypertension and aortic stenosis) should be treated aggressively and discontinuation of adriamycin at doses of 450 mg/m^2 should be considered in this group of patients. In addition, in Cortes' series (6), seven of 86 patients (8%) whose pre-adriamycin ECG was normal had CHF, while two of 14 patients (14%) whose ECG before adriamycin was abnormal had CHF. It is felt, therefore, that patients whose initial ECG is abnormal require close cardiac monitoring during adriamycin chemotherapy.

CURRENT RECOMMENDATIONS OF THE M. D. ANDERSON HOSPITAL

Adriamycin appears to be the best single agent for a number of malignant diseases, and in some diagnoses it is the only drug of therapeutic significance. We now have the knowledge to decrease the frequency and severity of cardiac toxicity.

1. Adriamycin must not be used indiscriminately. Strict adherence to a dose limitation is required. Our recommendation for most patients is a dose of 550 mg/m^2.
2. Restrict patients who received prior radiation therapy to the heart or concurrent cyclophosphamide or both to a dose of 450 mg/m^2.
3. Associated heart disease must receive aggressive medical management, especially hypertensive heart disease. In the latter case, patients should be limited to doses of 450 mg/m^2, and even further restriction may be necessary if the patients' hypertension cannot be well controlled.
4. Any new cardiovascular symptom or physical finding must be investigated thoroughly to rule out a cardiomyopathic etiology and to avoid administering even one additional dose of adriamycin to a patient with an already severely damaged heart.
5. Once a patient has received a full cumulative dose of adriamycin it should not be administered again.
6. The development of a decrease in the limb lead QRS voltage of 40% or greater by itself may be an indication to discontinue adriamycin. More information on this test is needed. Presently, we discontinue adriamycin if there is a 40% decrease in QRS voltage in conjunction with the development of any cardiac abnormality.
7. Adriamycin administered to adults either as an intermittent single dose or daily for 3 days appears to have the same dose limitation of 550 mg/m^2. It is possible that different guidelines may apply for other schedules and other age groups.
8. There is no test available yet which allows one to safely exceed the 550-mg/m^2 dose limit. Any experiment which calls for doses of adriamycin above this dose limit is hazardous, for it may result in cardiac toxicity leaving a severely incapacitated or dead patient. It is our opinion that unproven methods suggesting that this dose limitation be exceeded should first be proven in laboratory animals before being tried in man, and even then only in patients in whom the need for further adriamycin *far* exceeds the risk of cardiac toxicity.

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Adriamycin (NSC-123127) Cardiomyopathy[1,2]

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SUMMARY

The cardiotoxic effects of adriamycin were studied in 758 patients who were treated for far-advanced carcinoma. Seventy-seven patients (10.2%) exhibited transient electrocardiographic changes and 12 other patients developed severe congestive heart failure. Nine of these latter patients died within 3 weeks of the onset of the cardiac decompensation. The diffuse nature of this myocardiopathy was suggested by: *(a)* a conspicuous decrease in the QRS voltage on the electrocardiogram; *(b)* rapidly occurring cardiac dilatation and ventricular failure; and *(c)* refractoriness to inotropic drugs and mechanical ventricular assistance. Postmortem examination of the hearts of two patients showed a striking decrease in the number of cardiac muscle cells present, degeneration of the remaining myocardial cells, and mitochondrial distortion with dense inclusion bodies. Congestive heart failure occurred in only one of 717 patients (0.13%) who were treated with less than 550 mg/m^2 of adriamycin but there were 11 cases of cardiac failure in the 41 patients (26.8%) who received more than 550 mg/m^2 of this drug. Therefore, until more direct means are established to prevent adriamycin-induced cardiomyopathy, it is suggested that the total dose of adriamycin should be limited to less than 550 mg/m^2 to permit safer use of this efficacious cancer chemotherapeutic agent.

[Cancer Chemother Rep Part 3, vol 6:203-208, 1975]

Adriamycin is an antineoplastic drug with proven objective effectiveness in a variety of solid tumors and hematologic malignancies (1,2). It is a closely related analog of daunorubicin[8] which is an agent known to possess limiting cardiotoxic effects (3). Laboratory studies in which these two drugs were compared indicated that adriamycin had a wider therapeutic index than daunorubicin (4). These data, and the suggestion that adriamycin may be less cardiotoxic than its parent compound, led to extensive clinical trials in which adriamycin was utilized for a diverse group of neoplastic disorders. The most frequent manifestations of drug toxicity reported were alopecia, stomatitis, myelosuppression, and nausea (1). Cardiotoxic effects of the drug were noted less commonly, but cardiotoxicity occasionally manifested itself as fulminant congestive heart failure resulting in the premature death of a patient (2,5).

We have used adriamycin for the treatment of far-advanced cancer in 758 patients. During this time, 12 patients who had no prior history of cardiac disease experienced the sudden onset of severe congestive heart failure (table 1). Nine of these 12 patients died from cardiac decompensation within 3 weeks from the time that the symptoms of heart failure were first noted. Two patients had detailed postmortem examinations which included an electron microscopic study of the heart. The case histories and autopsy findings of these patients are described in this report.

Our purposes are: *(a)* to emphasize the differences between the isolated transient electrocardiographic (ECG) changes that occasionally occur during treatment with adriamycin and the syndrome of congestive heart failure; *(b)* to delineate the clinical and pathologic manifestations of the cardiomyopathy produced by adriamycin; and *(c)* to propose a total dose limitation which will hopefully prevent future irreversible myocardial damage by this effective cancer chemotherapeutic agent.

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[2]Adriamycin: CAS reg. No. 23214-92-8.

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[8]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

TABLE 1.—Clinical details of 12 patients with adriamycin-induced cardiomyopathy*

Patient No.	Age (yrs)/ sex	Diagnosis	Degree of tumor regression (%)	Total dose of adriamycin (mg/m^2)	Current status	Postmortem findings
1	83/M	Transitional cell carcinoma of bladder	>50	505	Dead	No autopsy
2	55/M	Adenocarcinoma of prostate gland	Subjective improvement	555	Dead	Dilated heart, fibrosis, myocytolysis
3	58/F	Clear cell carcinoma of kidney	>50	560	Dead	Dilated heart, myocytolysis
4	33/M	Lymphosarcoma	100	600	Alive	
5	52/F	Follicular carcinoma of thyroid gland	>50	620	Alive	
6	61/M	Papillary carcinoma of thyroid gland	>50	630	Alive	
7	24/F	Osteogenic sarcoma	>90	720	Dead	Dilated heart, myocytolysis
8	58/F	Squamous cell carcinoma of cervical skin	>50	755	Dead	No autopsy
9	58/M	Adenocarcinoma of prostate gland	>90	810	Dead	Dilated heart, myocytolysis
10	42/M	Leiomyosarcoma	>25	882	Dead	Dilated heart, myocytolysis
11	54/F	Hürthle cell carcinoma of thyroid gland	>90	895	Dead	No autopsy
12	68/M	Transitional cell carcinoma of bladder	>50	1041	Dead	No autopsy

*Originally published in part in *Cancer* (32:302-314, 1973).

FIGURE 1.—Normal chest roentgenogram (patient No. 7) before treatment (Oct 1970) with adriamycin (left) and development of cardiomegaly, a large right pleural effusion, and blunting of the left costophrenic angle after adriamycin therapy (720 mg/m^2, Nov 1971) (right). [Reproduced with permission from *Cancer* (32:302-314, 1973).]

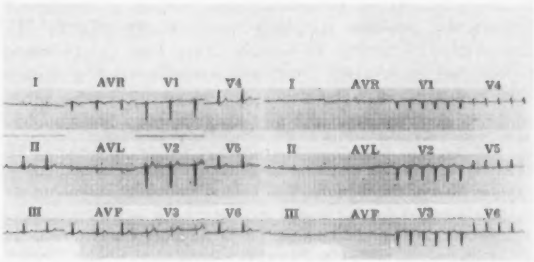

FIGURE 2.—Normal ECG prior to adriamycin (Feb 1971) (left) and a marked decrease in QRS voltage at the time of onset of the cardiomyopathy after adriamycin therapy (720 mg/m^2, Nov 1971) (right) in patient No. 7. [Reproduced with permission from *Cancer* (32:302-314, 1973).]

CASE REPORTS

Patient No. 7

A 23-year-old woman who had osteogenic sarcoma of the left sphenoid bone in February of 1970 was initially treated with 6600 rads of cobalt radiotherapy over an 8-week period. A local recurrence 4 months later was managed with intra-arterial infusions of actinomycin D (NSC-3053) (total dose, 2.0 mg). Another recurrence and severe proptosis necessitated enucleation and debridement of the left orbit

in March of 1971. Shortly thereafter, treatment was begun with adriamycin given at a dose of 60 mg/m^2 intravenously (iv) every 3 weeks. During the subsequent 9-month period she received 1112 mg (720 mg/m^2) of this drug. Periodic radiographic examinations of the paranasal sinuses revealed progressive regression in the size of the tumor. Chest roentgenograms, ECGs, and determinations of serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), and creatine phosphokinase (CPK) levels were taken monthly. The results of these tests were normal.

On November 29, 1971, the patient was admitted to the hospital with a 1-week history of severe dyspnea, fatigue, and a dry cough. Her blood pressure was 85/50 mm Hg, her pulse rate was 120, and her respiratory rate was 25. There was a loud mid-diastolic gallop sound. The chest roentgenogram revealed cardiomegaly and bilateral pleural effusions (fig 1). The ECG showed a marked diminution in QRS voltage in all leads as compared to previous tracings (fig 2). The SGOT, LDH, and CPK levels were still within the normal range.

Treatment was begun with digoxin and furosemide but the hypotension persisted and she became confused and oliguric. The mean pulmonary capillary wedge pressure was 23 mm Hg and the A-V_{02} difference was 8.0 vol% reflecting the profound ventricular failure and reduced cardiac output. Intra-aortic balloon pumping with counterpulsation was instituted in an effort to improve the cardiocirculatory status (6). Despite this type of mechanical ventricular assistance, as well as continued digitalis administration and repeated trials with iv isoproterenol, levarterenol, metaraminol, calcium gluceptate, and glucagon, the patient's cardiac decompensation did not improve and she died on December 9, 1971.

Autopsy

At autopsy it was found that the heart weighed 250 g and was dilated; the coronary arteries were normal; the lungs revealed massive pulmonary edema; and the primary osteogenic tumor was necrotic.

Sections of the myocardium that were examined by light microscopy showed a marked decrease in the number of muscle fibers and an increase in the amount of interstitial tissue (fig 3). The loss of cells resulted in the disruption of the "syncytial" pattern seen in normal myocardium. Electron microscopic examination of the myocardium showed degeneration of the myofibrils, Z-line irregularity, and swelling of the mitochondria (fig 4). Many of the enlarged mitochondria contained dense deposits. No glycogen granules were visualized in any of the muscle cells examined with the electron microscope.

Patient No. 9

A 58-year-old man was referred to the M.D. Anderson Hospital and Tumor Institute for management of painful vertebral and pelvic osteoblastic metastases from carcinoma of the prostate gland. Prior

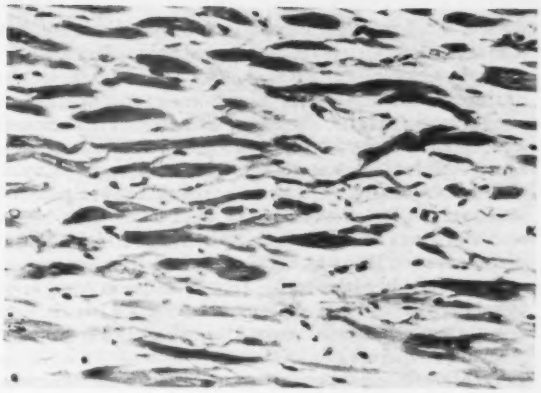

FIGURE 3.—Decrease in No. of cardiac muscle fibers and a relative increase in the interstitial tissue in patient No. 7 (H & E stain, × 300).

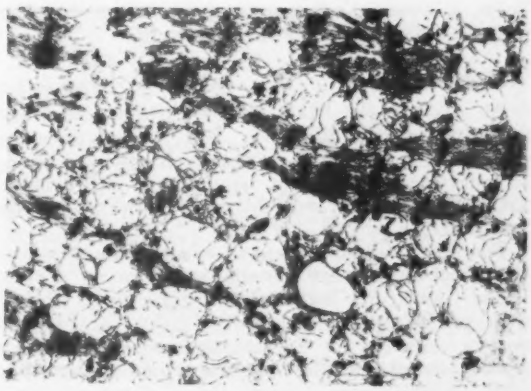

FIGURE 4.—Remnants of the myofibrils exhibit widened and disrupted Z lines; the mitochondria are vacuolated and contain disorganized cristae and prominent electron-dense bodies (× 30,000).

therapy had included a transurethral resection of the prostate gland, 5000 rads of radiotherapy, bilateral orchiectomy, and daily administration of 3 mg of diethylstilbestrol.[8] There had been no previous history of cardiopulmonary disease and the patient had not had any symptoms suggestive of cardiac dysfunction at that time. He initially received adriamycin chemotherapy at an iv dose of 60 mg/m^2. An evaluation after 225 mg/m^2 was given revealed that the bone pain had completely disappeared and the previously elevated serum alkaline phosphatase level had returned to normal. He was able to resume employment as an automobile mechanic. After 500 mg/m^2 was given, the bone roentgenograms demonstrated marked improvement at the sites of the metastatic lesions. Therapy with adriamycin was continued until November 11, 1971, at which time a total

[8]Diethylstilbestrol: NSC-3070; 4,4'-stilbenediol,α,α'-diethyl-; stilbestrol.

dose of 1785 mg (825 mg/m^2) was given. Serial ECGs and serum enzyme levels remained normal.

Two weeks after the last dose of adriamycin the patient noted the onset of dyspnea on exertion, a cough, and fatigue. Physical examination revealed a blood pressure of 90/70 mm Hg, a pulse rate of 125, and a respiratory rate of 38. The jugular veins were visible at the angle of the mandible when the patient was seated. Rales were heard over both lung bases and there was an audible third heart sound. The ECG demonstrated sinus tachycardia, left-axis deviation, and a marked reduction in QRS voltage as compared to a tracing performed 11 months earlier (fig 5). The chest roentgenogram showed cardiomegaly and bilateral pleural effusions (fig 6).

Treatment was begun with digoxin, furosemide, and supplemental oxygen but the degree of congestive heart failure did not improve. The severe hypotension and oliguria were completely refractory to isoproterenol and metaraminol. The patient died on December 11, 1971, ie, 2 weeks after his first symptoms of heart failure were noted and 1 month after he received the last dose of adriamycin.

Autopsy

At autopsy the heart weighed 540 g and there was biventricular dilatation. The coronary arteries exhibited areas of focal eccentric narrowing by atherosclerotic plaques, but none of the three major arteries were obstructed by more than 25% of the total lumen diameter.

There was only one focus of microscopic metastatic adenocarcinoma in a lumbar vertebra.

The edematous myocardial intercellular spaces were prominent because of the diffuse absence of muscle cells (fig 7). Remaining myocardial fibers demonstrated granular and vacuolar degeneration with nuclear pleomorphism. Cross striations were not visible in the degenerating cells. Electronmicrographs showed disrupted myofilaments intermingled with degenerating Z bands. Many mitochondria were enlarged and contained disorganized cristae and prominent electron-dense bodies. The sarcoplasmic reticulum was dilated in the degenerating fibrils with clumping and margination of the chromatin in the nuclei of these cells.

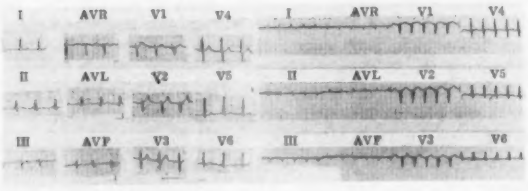

FIGURE 5.—Normal ECG before adriamycin therapy (Nov 1970) (left) and decreased QRS voltage after treatment (810 mg/m^2, Nov 1971) (right) in patient No. 9. [Reproduced with permission from *Cancer* (32:302-314, 1973).]

FIGURE 6.—Normal chest roentgenogram during adriamycin treatment (Sept 1971) (left) and cardiomegaly with bilateral pleural effusions (right) after therapy (810 mg/m^2, Nov 1971) in patient No. 9. [Reproduced with permission from *Cancer* (32:302-314, 1973).]

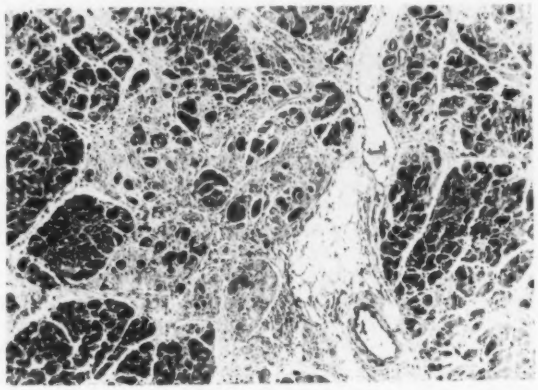

FIGURE 7.—Degeneration of myocardial cells and prominence of interstitial tissue in patient No. 9 (H & E stain, × 1500).

DISCUSSION

The cardiac abnormalities produced by adriamycin may be separated into two categories for analysis: (*a*) ECG alterations and (*b*) congestive heart failure. Both of these manifestations of drug-induced cardiotoxicity mimic those that have been reported during therapy with daunorubicin (3, 7, 8). Seventy-seven of the 758 patients (10.2%) that we have treated with adriamycin exhibited ECG changes. These abnormalities were not specific and were noted with equal frequency at all total dose levels. They consisted of supraventricular tachyarrhythmias, occasional ventricular ectopic beats, S-T segment depression, T-wave flattening, and left-axis deviation. These changes have been observed during the iv administration of adriamycin or daunorubicin and in the course of chronic treatment programs with either of these drugs. A resumption to the original ECG pattern usually occurred shortly after discontinuation of the chemotherapy. None of the patients who demonstrated ECG alterations subsequently experienced congestive heart failure but, frequently, adriamycin was discontinued when these changes first occurred.

The two patients presented in this report exemplified the second manifestation of adriamycin cardiotoxicity, ie, congestive heart failure. The outstanding clinical feature of this acute cardiac decompensation was the insidious onset of symptoms after which rapidly progressive biventricular failure and death occurred. This profound cardiac dysfunction was also marked by total refractoriness to cardiotonic drugs and mechanical circulatory assistance. Of the nine patients who died of adriamycin cardiomyopathy, none survived longer than 3 weeks after the onset of congestive heart failure.

The conspicuous diminution in QRS voltage that occurred in these patients during treatment with adriamycin suggested the diffuse character of the myocardial damage. The diagnostic usefulness of this ECG finding, however, was lessened by its lack of specificity since this change also has been described in patients with pericardial or pleural effusion, myocarditis, severe coronary artery disease, other types of cardiomyopathy, and myocardial infiltrative processes, eg, metastatic disease of the heart, hemachromatosis, and amyloidosis (9,10). Serum enzyme elevations occurred late in the course of adriamycin-induced cardiac failure and, therefore, these changes were not helpful for making an early diagnosis of the myocardiopathy.

The pathologic findings in our two patients confirmed the clinical impression of diffuse cardiac injury. The principal abnormalities observed were a marked decrease in the number of muscle fibers and mitochondrial distortion. The muscle loss or myocytolysis that occurred and the mitochondrial abnormalities are both nonspecific responses of cardiac muscle to injury that have been described in a variety of cardiomyopathies, including those produced by daunorubicin (11-14).

In an effort to limit the incidence of future cardiac complications with adriamycin, we examined the relationship between the total dose of this drug administered to 758 patients and the incidence of subsequent heart failure (table 2). This analysis revealed that the cardiomyopathy occurred in only one of 717 patients (0.13%) who received less than 550 mg/m^2 (range, 45-545 mg/m^2) of adriamycin. However, of the 41 patients who were treated with more than 550 mg/m^2 of adriamycin (range, 555-1040 mg/m^2), severe cardiac failure occurred in 11 patients (26.8%). Eight of these latter 11 patients died from refractory congestive heart failure.

TABLE 2.—Relationship of adriamycin cardiomyopathy to total dose*

Total cumulative dose of adriamycin (mg/m^2)	No. of patients	No. of patients with congestive heart failure
≤450	663	0
451-500	23	0
501-550	31	1
551-600	14	3
≥601	27	8

*Originally published in part in *Cancer* (32:302-314, 1973).

Investigations to determine the etiology of adriamycin cardiotoxicity and to detect its negative inotropic effects before heart failure becomes overt are being performed in a number of centers (15, 16). Preliminary evidence suggests that the aglycone metabolite of adriamycin may be the agent responsible for the myocardial dysfunction (17). Efforts to interfere with the acute cardiotoxic effects of this metabolite have been successful in isolated canine preparations with agents such as ethylenediaminetetraacetic acid and ICRF-159 (15).[9] Kobayashi et al have shown that arrhythmia induction by adriamycin may be blocked by *dl*-propanolol in experimental animals (18). It is anticipated that future studies such as these will lead to the development of more specific means to prevent the cardiac side effects of adriamycin without interfering with its antineoplastic activity. Until that time, though, limitation of the total dose of adriamycin to less than 550 mg/m^2 should minimize the occurrence of serious cardiotoxicity and thus permit the safer utilization of this effective chemotherapeutic agent.

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Cardiotoxicity of Adriamycin (NSC-123127) in Children[1,2]

Angela C. Gilladoga, Corazon Manuel, Charlotte C. Tan, Norma Wollner, and M. Lois Murphy[3,4]

SUMMARY

Of 50 children who received adriamycin in cumulative doses of 495-1695 mg/m², eight (16%) had severe cardiomyopathy with congestive heart failure. The incidence was higher in those patients who also had incidental cardiac radiation. Of 68 patients who received 200-495 mg/m² of adriamycin, none had cardiomyopathy. Heart failure was controlled in all children. One patient with cardiomyopathy had a fatal pulmonary infarction secondary to thromboemboli arising from the heart. With few exceptions, electrocardiograms provided the first indication of cardiac disturbance in these children although the electrocardiographic abnormalities associated with cardiomyopathy appeared late. Early transient electrocardiographic changes did not predict which patient would experience cardiomyopathy. At present, dose limitation is the only way to prevent adriamycin cardiomyopathy. Our recommendations are discussed.

[Cancer Chemother Rep Part 3, vol 6:209-214, 1975]

Our experience with the cardiotoxic effects of adriamycin in children with malignancy who were treated at our hospital is presented.

ELECTROCARDIOGRAMS (ECGs)

With few exceptions, the ECG provides the first indication of cardiac disturbance in children receiving chemotherapeutic agents. A retrospective review (1) of the ECGs performed for 23 children who had received 12-55 mg/kg of adriamycin over a 2-20 month period showed that six (25%) children had flattening of the T waves, prolonged QT interval, and/or decreased QRS voltages. None had received daunorubicin[5] previously. These changes were nonspecific and transient except in a 4-year-old girl who had heart failure at a total cumulative dose of 24.5 mg/kg.

To study the effects of adriamycin administration on the ECG and to determine whether early ECG changes would predict which patients will exhibit cardiomyopathy, ECGs were obtained before the first adriamycin dose (Day 0), within 1 hour after the second and third doses (Days 1 and 2), and 1 week after an adriamycin course (Day 9) for 43 children ranging in age from 21 months to 15 years. Ten of these 43 children were also studied during actinomycin D administration.[6]

Seven (16%) children receiving cumulative doses of 75-370 mg/m² of adriamycin had transient ECG changes consisting of mild flattening of the T waves (four patients) and marked sinus tachycardia (two patients). These changes appeared during the first to fifth courses on Days 1 and 2 and were no longer present on Day 9. The seventh patient had severe sinus bradycardia to 40 beats/minute with hypotension 2 weeks after her first adriamycin course consisting of 30 mg/m²/day × 3. Her heart rate returned to normal within 4 days and sinus bradycardia did not recur on subsequent adriamycin courses of 20 mg/m²/day × 3. Serum electrolytes, serum creatine phosphokinase, and serum glutamic oxaloacetic transaminase (SGOT) levels were normal in all children. One of the ten patients studied during actinomycin D administration had mild T-wave flattening.

These early ECG changes did not predict which patients would experience cardiomyopathy. Of the seven patients who had transient ECG changes, two subsequently had adriamycin cardiomyopathy at cumulative doses of 720 and 810 mg/m². However, four of 36 patients who had no ECG changes experienced adriamycin cardiomyopathy at 495, 590, 905, and 1695 mg/m².

ECG abnormalities associated with cardiomyopathy appeared late. These consisted of progressive flattening to inversion of the T waves in Leads I and V_{5-6} (fig 1). These findings warned us of impending heart failure in five patients who had adriamycin cardiomyopathy.

We now obtain ECGs before each course of adriamycin. Because frequent premature ventricular con-

[1]Supported by Public Health Service grant CA-05826 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, and the Judith Harris Selig Memorial Fund, Inc.

[2]Adriamycin: CAS reg. No. 23214-92-8.

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[4]*Reprint requests to:* A. C. Gilladoga, MD, Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

[5]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6; daunomycin.

[6]Actinomycin D: NSC-3053; dactinomycin.

tractions occurred in three children, first-degree atrioventricular block occurred in three, and significant ST-T abnormalities occurred in seven, adriamycin was discontinued in four patients receiving doses of 600, 630, 780, and 825 mg/m^2 and was limited to 500 mg/m^2 in nine patients. We cannot be certain whether these ECG changes were due to toxic drug effects or infections.

ADRIAMYCIN CARDIOMYOPATHY

Fifty children, ranging in age from 8 months to 15 years when the first adriamycin dose was administered, reached cumulative doses exceeding 500 mg/m^2 between September 1971 and August 1974. These children were observed for periods of 11-57 months. Adriamycin was given over an 8-31-month period and has been discontinued in 36 patients from 12 to 27 months.

The adriamycin dose schedule in 28 patients with solid tumors consisted of two courses of 20 mg/m^2/day × 3 of adriamycin given intravenously (iv) at 2-week intervals followed by actinomycin D, vincristine, and cyclophosphamide[7] (2,3). The cycle was repeated every 3 months. Thirteen patients received monthly adriamycin courses of 30 mg/m^2 on Days 1 and 2 and 15 mg/m^2 on Day 3 or 45 mg/m^2/day × 2 at 2 weeks after high-dose methotrexate with citrovorum factor rescue (4).[8] Nine patients had variable dose schedules.

The incidence of cardiomyopathy in these 50 patients was 16%: four boys and four girls aged 2-14 years at the onset of heart failure which occurred at cumulative adriamycin doses of 495 (plus 120 mg/kg of daunorubicin) to 1695 mg/m^2 given over periods of 9-29 months. Four children also had incidental cardiac radiation.

Analysis of 42 patients who received over 500 mg/m^2 of adriamycin with no incidental cardiac radiation therapy (group 1) versus eight patients who, in addition, received 1000-5000 rads of therapy to the lungs and/or mediastinum given at 150-250 rads daily before or during adriamycin administration (group 2),showed a 50% incidence of severe cardiomyopathy in group 2 (P=0.02) as compared to 9.5% in group 1 (table 1). Furthermore, heart failure occurred at lower doses of adriamycin in group 2 (table 2).

The incidence of cardiomyopathy in group 1 increased at higher cumulative doses (P=0.05). Of 21 patients who received 501-600 mg/m^2 none had cardiomyopathy; however, one of 11 (9%) at a dose of 601-800 mg/m^2, two of seven (29%) at a dose of 801-1000 mg/m^2, and one of three (33%) at a dose of 1001-1695 mg/m^2 did have cardiomyopathy (table 3).

Sixty-eight patients received cumulative doses of adriamycin at 200-495 mg/m^2 and have been observed for a period of 8-60 months; of these patients eight had adriamycin discontinued for 8-39 months after receiving 285-495 mg/m^2. None of these 68 have cardiomyopathy.

Patients with severe adriamycin cardiomyopathy showed ECG evidence of myocardial damage 6 weeks before their last adriamycin dose (patient No. 1) to within 14 weeks later (patient Nos. 2-8), coinciding with heart failure in three patients (Nos. 3, 5, and 7). Five patients had cardiac decompensation 3-13 weeks later.

Congestive heart failure was well controlled in six patients for 4-32+ months, seven patients required prolonged digitalis therapy, and four patients continued on diuretics beyond 4 months. Subsequently, five patients died: patient Nos. 1, 6, and 7 from malignancy, patient No. 3 from pulmonary thromboembolism, and patient No. 4 from an undetermined cause.

In patient No. 1, control of heart failure was excellent for 26 months. He was the only patient in whom we were able to discontinue digitalis without recurrence of heart failure. He received digitalis for 18 months. His heart returned to normal size as seen on a chest roentgenogram and his ECG was much improved. He died of diffuse metastatic Ewing's sarcoma. Unfortunately, no autopsy was performed.

Patient No. 2 is our longest followup patient (32+ months). Digitalis was discontinued after 12 months but was resumed 2 weeks later. Presently, heart failure is well controlled and his heart is still moderately enlarged on the chest roentgenogram with a dilated and poorly contractile left ventricle on the echocardiogram. There is persistent ECG evidence of

[7]Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,-2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

[8]Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

Citrovorum factor: NSC-3590; CAS reg. No. 6035-86-5; glutamic acid, *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-, calcium salt

TABLE 1.—Comparative incidence of cardiomyopathy in 50 children who received >500 mg/m^2 of adriamycin with or without incidental cardiac radiation

	No. of patients	Incidental cardiac radiation (rads)	Incidence of cardiomyopathy* (No. of patients/%)
Group 1	42	0	4/9.5
Group 2	8	1000-5000	4/50
Totals	50		8/16

*P = 0.02.

TABLE 2.—Clinical data on 8 children with adriamycin cardiomyopathy

Patient No.	Age (yrs)/ sex	Malignant diagnosis	Incidental cardiac radiation	Dose of adriamycin		ECG abnormalities—*		CHF		Status
				Mg/m²	Mos	From last adriamycin dose (wks)	To onset of CHF (wks)	Control	Mos	
1	12/M	Ewing's sarcoma, pubis	—	1695	22	-6	13	Good	26	Died of metastatic Ewing's sarcoma; no autopsy
2	14/M	Ewing's sarcoma, mandible	—	905	14	14	5	Good	32+	Alive, receiving digitalis; no evidence of malignant disease
3	11/F	Malignant teratocarcinoma, ovary	—	810	22	8	0	Fair	6	Died of pulmonary thromboemboli; autopsy showed mural thrombi, right and left ventricles; no evidence of malignant disease
4	10/M	Osteogenic sarcoma, humerus	—	675	11	0	9	Good	4	Sudden death, arrhythmia (?); autopsy showed metastatic osteogenic sarcoma, adriamycin cardiomyopathy
5	7/M	Ewing's sarcoma, tibia	2100 rads to mediastinum 1 wk before adriamycin	720	15	2	0	Fair	20+	Alive, receiving digitalis and diuretics; no evidence of malignant disease
6	11/F	Osteogenic sarcoma, humerus	1400 rads to left lung 1 mo before adriamycin	600	9	6	3	Fair	6	Died of metastatic osteogenic sarcoma; no autopsy
7	2/F	Embryonal adenocarcinoma, ovary	1200 rads to mediastinum/ pulmonary area during adriamycin	590	13	5	0	Good	12	Died of metastatic embryonal adenocarcinoma; autopsy showed endocardial fibrosis
8	11/F	Hodgkin's disease	3500 rads to mediastinum 1 yr before adriamycin	495†	29	8	3	Good	23+	Alive, receiving digitalis; no evidence of malignant disease

*CHF = congestive heart failure.
†Plus 120 mg/m² of daunorubicin.

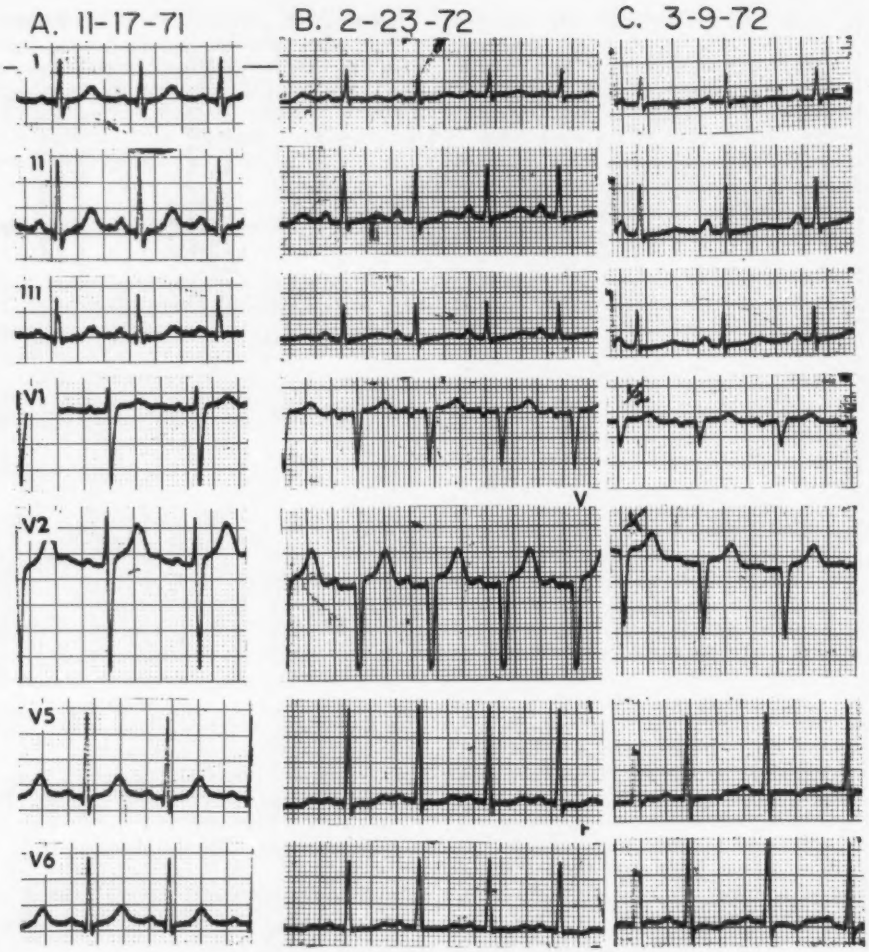

FIGURE 1.—ECG abnormalities (patient No. 2) seen in adriamycin or daunorubicin cardiomyopathy. A. A normal tracing after last adriamycin course (total dose, 905 mg/m^2) given over 14 mos. B. Flattening of T waves (I, II, III, V_{5-6}), loss of R voltage in V_{1-2}, and intraventricular conduction defect 14 wks later. C. Progressive flattening to inversion of T waves (I, II, III, V_{5-6}) 3 wks before onset of heart failure.

myocardial damage. The patient no longer receives chemotherapy and has no evidence of Ewing's sarcoma. He is attending school and only strenuous activities are restricted.

In patient Nos. 3 and 5, heart failure was difficult to control. Both of these patients were receiving prolonged digitalis and diuretic therapy. Mitral insufficiency murmur appeared in both patients within 2 months after the onset of heart failure. Patient No. 3 died 6 months later from massive pulmonary infarction. An autopsy showed that her heart was dilated with normal mitral valve, there were mural thrombi in both ventricles, and there was no evidence of embryonal adenocarcinoma. Microscopy showed diffuse myocardial damage consistent with adriamycin cardiomyopathy.

TABLE 3.—Cumulative dose and incidence of cardiomyopathy in 42 children receiving > 500 mg/m^2 of adriamycin without incidental cardiac radiation—group 1

No. of patients	Dose of adriamycin (mg/m^2)	No. of patients with cardiomyopathy*/%
21	501-600	0/0
11	601-800	1/9
7	801-1000	2/29
3	>1000	1/33
42		4/9.5

*$P = 0.05$.

Patient No. 5 is alive at 20+ months after the onset of heart failure and is currently receiving digitalis and diuretics. He has persistent gallop rhythm. A chest roentgenogram showed that his heart is not enlarged; however, an echocardiogram showed a dilated and poorly contractile left ventricle. He is attending school but ordinary activities such as climbing stairs are restricted. He is no longer receiving chemotherapy and has no evidence of Ewing's sarcoma.

Heart failure was well controlled in patient No. 4 for 4 months. He died unexpectedly of an undetermined cause. Twenty-four hours after he received high-dose methotrexate with citrovorum factor rescue, he experienced acute respiratory distress at home after drinking orange juice and was dead on arrival at a nearby hospital. An autopsy done at our hospital showed no evidence of aspiration but there was pulmonary metastatic osteogenic sarcoma. Adriamycin cardiomyopathy was confirmed on microscopy. A fatal cardiac arrhythmia cannot be ruled out.

Although patient No. 6 had well-controlled heart failure for 6 months, she required continued digitalis and diuretic therapy. She died of extensive pulmonary metastatic osteogenic sarcoma. No autopsy was performed.

Patient No. 7 had well-controlled heart failure for 12 months. A chest roentgenogram showed her heart had returned to its normal size and ST-T abnormalities on the ECG were much improved. She died of diffuse metastatic embryonal adenocarcinoma. An autopsy showed endocardial fibrosis; we do not know whether this represents healed adriamycin cardiomyopathy.

Patient No. 8 is alive at 23+ months after the onset of heart failure. Digitalis was discontinued after 12 months but restarted 1 week later. Heart failure is presently under good control. Her ECG showed intermittent Wolff-Parkinson-White syndrome with no tachycardia. During normal conduction, ST-T abnormalities appeared much improved. Her chest roentgenogram showed a normal size heart; however, the echocardiogram showed that the left ventricle is dilated and poorly contractile. She is not receiving chemotherapy and has no evidence of Hodgkin's disease. She is in school and only strenuous activities are restricted.

DIAGNOSIS AND MANAGEMENT

The incidence of congestive heart failure after prolonged adriamycin treatment is presumed to be due to cardiomyopathy when: (*a*) there are ECG ST-T abnormalities consistent with myocardial damage, and (*b*) a large poorly contractile heart is seen on a fluoroscopy or echocardiography in a patient with a previously normal heart who has no known history of heart disease and no evidence of viral or rheumatic carditis. Serum creatine phosphokinase and SGOT determinations have not been helpful. Cardiac biopsies have not been done in any of our patients. The decreased thickness of the myocardium and the presence of mural thrombi are added risks to this procedure.

The treatment of congestive heart failure consists of prolonged digitalis and diuretic therapy and a low salt diet. Activities should be restricted with a gradual return to normal in 4 months. These patients are extremely sensitive to digitalis therapy and require a total iv digitalizing dose (TDD) of only 0.010-0.020 mg/kg. The maintenance dose is 1/4-1/7 digoxin TDD or 1/10-1/15 digitoxin TDD. Nausea, vomiting, abdominal pains, bradycardia, and tachycardia are the usual manifestations of digitalis toxicity that we have encountered.

The early detection of adriamycin cardiotoxicity, however, is essential for the successful management or prevention of cardiomyopathy. We are presently studying the application of systolic time-interval measurements and echocardiography to measure cardiac function in these patients. By echocardiography, we can determine left ventricular indices previously obtainable only by cardiac catheterization and angiocardiography (5-8). Recently, it has been reported that early adriamycin cardiotoxicity can be detected by measurements of systolic time intervals (9).

RECOMMENDATIONS

Based on these observations, we recommend the following maximum cumulative doses of adriamycin in children.

A. In patients who receive incidental cardiac radiation we suggest 500 mg/m^2. Our clinical experience indicates that incidental cardiac radiation increases the cardiotoxicity of adriamycin. Preliminary studies in rabbits show that adriamycin at doses not known to be cardiotoxic caused progressive myocardial damage on the ECG with clinical deterioration not seen in rabbits receiving radiation or adriamycin alone.[9]

B. In patients with existing cardiac abnormalities or severe pulmonary disease we suggest 500 mg/m^2. For instance, when there are frequent premature ventricular contractions, there should be improvement in the ECG before the drug is administered. These ECG abnormalities may indicate existing heart disease from other causes such as infection. Administration of a cardiotoxic agent to such patients may lead to serious arrhythmia or cardiac decompensation.

C. In all other patients, 600 mg/m^2 is recommended. Our previous maximum limit in this group was 800 mg/m^2. However, we do not know if patients with normal ECGs do not have cardiomyopathy. We are hoping that we will be able to obtain more precise information about myocardial function in our patients by echocardiography. There is evidence in

[9]Tokita N, Gilladoga AC, John M, et al. Increased cardiotoxicity in rabbits given radiation and adriamycin. Unpublished work.

rabbits[10] that significant ECG abnormalities are generally associated with severe myocardial damage. However, moderately severe lesions were also seen with mild nonspecific T-wave changes.

It also seems prudent that these agents should be given at the lowest chemotherapeutically effective dose. We recommend divided dose schedules of adriamycin such as 20 mg/m^2/day × 3 or 15 mg/m^2/day × 5 (1-3). In preliminary studies involving small groups of rabbits there was some suggestion that adriamycin given in divided doses was less cardiotoxic.[10] It did not prevent adriamycin cardiomyopathy but the clinical disturbance was less severe. Larger groups of animals should be studied to determine whether divided dose schedules produce less severe cardiac lesions.

We also recommend that ECGs be obtained before adriamycin administration, particularly when cumulative doses over 300 mg/m^2 have been reached. With its limitations, the ECG can be helpful. The application of systolic time-interval measurements and echocardiography in the early detection of cardiotoxicity is currently under investigation.

There have been some reports indicating that pretreatment with digitalis may prevent adriamycin cardiomyopathy (10). At this time, we are not employing this method because our experimental studies failed to confirm these observations.[10] Pretreatment with digitalis did not prevent the development of adriamycin cardiomyopathy in rabbits. Furthermore, the clinical disturbances were as severe as in the undigitalized rabbits on single-dose schedules. Cardiac tissue assay in rabbits and rats pretreated with maximally tolerated doses of Ouabain, Strophantin K, and digitoxin showed no decrease in adriamycin uptake.[11]

CONCLUSIONS

At present, dose limitation is the only way to prevent adriamycin cardiomyopathy. In most reported cases, congestive heart failure occurred at cumulative doses of more than 500 mg/m^2 of adriamycin (1, 11,12). Age, preexisting heart disease (such as from radiation or infection), severe pulmonary disease, dose schedules, administration of other potentially cardiotoxic agents such as anhydro-ara-5-fluorocytosine,[12] high-dose cyclophosphamide, or probably even high-dose methotrexate, may contribute to cardiac decompensation at lower doses of adriamycin.

Our recommendations are simply guidelines. One may give more or less drug depending on observed incidence of severe cardiotoxicity. By careful cardiac monitoring, congestive heart failure can be recognized early and managed successfully. Ultimately, the physician must determine whether the expected chemotherapeutic gain from administration of this agent outweighs the risk of serious cardiac complications.

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[11]Philips FS. Unpublished report.

[12]Anhydro-ara-5-fluorocytosine: NSC-166641; CAS reg. No. 40505-45-1; 6*H*-furo[2',3':4,5]oxazolo[3,2a]pyrimidine-2-methanol, 2-fluoro-3, 3a, 9a-tetrahydro- 3 - hydroxy - 6 - imino-, [2*R*-(2α, 3β, - 3aβ, 9aβ)]-, monohydrochloride.

Adriamycin (NSC-123127) Cardiotoxicity: A Clinicopathologic Correlation[1,2,3]

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SUMMARY

Adriamycin is an effective antitumor agent. It is known to produce cardiotoxicity which limits its prolonged use. In a phase I study of adriamycin (20 mg/m²/day × 4 up to 35 mg/m²/day × 3 repeated every 28 days) in 100 adults with cancer, a clinicopathologic correlation of adriamycin cardiotixicity was made. Two types of cardiotoxicity were noted. The first was early or immediate cardioxicity which consisted of (*a*) transient or reversible nonspecific electrocardiographic (EKG) abnormalities seen in 25% of the patients, such as ST-T changes, prolonged Q-T interval, premature atrial and ventricular beats, and atrial flutter and fibrillation; and (*b*) toxic cardiopathy such as interstitial edema between myocardial fibers, myocytolysis, and disruption of sarcoplasm with or without vacuole formation. Either of these findings was seen in the autopsies of 12 of 46 patients (26%) without congestive heart failure (CHF). The second type of cardiotoxicity was late or cumulative consisting of (*a*) nonspecific EKG changes such as nonreversible low R-wave voltage and poor R-wave progression in chest leads which preceded the CHF in six patients and the reversible arrhythmias; and (*b*) CHF with gross biventricular dilatation in four of five patients and toxic cardiopathy on microscopy in five of five patients who had autopsies. CHF was seen in none of 75 patients, two of 90 patients (2.2%), and seven of ten patients (70%) after less than 450, less than 600, and greater than 600 mg/m² of adriamycin respectively. CHF in six of seven patients responded to conventional therapy. In a phase II study of adriamycin in childhood solid tumors (15 mg/m²/day×4 up to 30 mg/m²/day×3 repeated every 21 days), CHF was seen in two of 150 patients at 450 and 500 mg/m². Both patients had prior radiotherapy to the mediastinal area. No CHF was seen in 44 patients with primary osteosarcoma treated with adriamycin (30 mg/m²/day×3 repeated every 28 days×6) at a cumulative dose less than 550 mg/m². Therefore, adriamycin, although an active antitumor agent, must be administered below its cardiotoxic threshold of 600 mg/m². The adriamycin-induced CHF is treatable and, therefore, vigorous support to reverse this toxicity deserves a trial.

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[2]This study was done by the Acute Leukemia Group B.

[3]Adriamycin: CAS reg. No. 23214-92-8; doxorubicin; ADM. Supplied by Farmitalia, Milan, Italy, through Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

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Adriamycin, a cytotoxic antibiotic, has been proven to be effective in malignant lymphomas, acute leukemias, sarcomas, and carcinomas of the breast, lung, thyroid, testes, and urinary bladder (1). Its major transient toxic effects include myelosuppression, stomatitis, alopecia, nausea, and vomiting. Despite its effectiveness, the serious cardiotoxicity of adriamycin limits its prolonged use (2-6).

The objectives of the present report are: (*a*) to determine the incidence of electrocardiographic (EKG) changes and congestive heart failure (CHF) resulting from adriamycin administration; (*b*) to correlate the adriamycin cumulative dose with the EKG changes and CHF; (*c*) to determine the reversibility of adriamycin-induced CHF; and (*d*) to correlate changes of the heart found at autopsy by gross examination and light microscopy with the cumulative dose of adriamycin and with the presence of CHF.

MATERIALS AND METHODS

The criteria for entry in this study included histologically proved cancer that was inoperable (except in the adjuvant study of primary osteosarcoma) for which no standard form of therapy existed. Laboratory requirements were a minimum leukocyte count of 4000 cells/mm^3, a minimum platelet count of 100,000 cells/mm^3, and blood urea nitrogen and creatinine levels less than 25 and 1.5 mg/100 ml respectively.

Hematologic, biochemical, and EKG examinations, chest roentgenogram, tomography, and bone survey were conducted before, during, and after therapy as necessary to check for possible tumor response and toxicity from the drug.

The criteria for an objective response are reported elsewhere (7).

Drug Dosage and Schedule

Each vial of adriamycin was diluted with 5 ml of sterile saline, and the calculated dose was injected as an intravenous bolus.

Initial Study

In a phase I study of adriamycin at Roswell Park Memorial Institute, 127 adults with advanced cancer were treated with adriamycin at the following dose schedules: (*a*) 20 mg/m^2/ day × 4 days; (*b*) 30 mg/m^2/ day × 3 days; and (*c*) 35 mg/m^2/day × 3 days.

Selection for study was nonrandom. Each schedule was repeated at intervals of 4 weeks if hematologic toxic effects had cleared.

Cardiotoxicity after adriamycin administration is defined as either: (*a*) nonspecific EKG changes (excluding sinus tachycardia<120/minute); (*b*) cardiac abnormalities on gross or microscopic examination at autopsy other than findings suggestive of either arteriosclerotic heart disease, myocardial infarct, or neoplastic infiltration of the myocardium; or (*c*) development of CHF in the absence of pre-existing symptomatic primary heart disease, or cor pulmonale from pulmonary metastases or secondary to neoplastic myocardial infiltration.

Criteria for evaluation of cardiotoxicity required two or more EKG examinations at least 1 month apart during therapy. If the patient died, a repeat EKG 3 weeks after the start of adriamycin qualified him for cardiotoxicity evaluation. In none of the patients who died before 3 weeks was cardiotoxicity considered to be the cause of death.

Phase II Study

In a phase II study of adriamycin in childhood solid tumors by the Acute Leukemia Group B, 150 children with advanced cancer were treated with adriamycin at two dose schedules (*a* or *b*) chosen at random. Subsequently, schedule *a* was dropped and random allocation to schedule *b* or *c* was undertaken. The doses were as follows:*(a)* 15 mg/m^2/day × 4 days; *(b)* 20 mg/m^2/day × 4 days; or *(c)* 30 mg/m^2/day × 3 days.

Each dose schedule was repeated at intervals of 3 weeks if hematologic toxic effects had cleared. The total cumulative dose of adriamycin in this study was limited to 600 mg/m^2.

Cardiotoxicity evaluation in this group was determined by the start of CHF or by EKG abnormalities seen in a few patients who had serial tracings.

Adjuvant Study of Primary Osteosarcoma

In the adjuvant study of amputation and adriamycin in primary osteogenic sarcoma, adriamycin was scheduled to be given after wound healing was complete. Forty-four patients were evaluated in this study. The dose was 30 mg/m^2/day for 3 successive days, repeated every 4-6 weeks for six courses. The median onset of adriamycin treatment for the entire group was 2 weeks after surgery. The total cumulative dose of adriamycin was 540 mg/m^2 over 5-7 months. Thereafter, patients remained untreated.

Cardiotoxicity in this group was determined by the onset of CHF.

RESULTS

At the Roswell Park Memorial Institute 127 adults with various cancers were treated with adriamycin alone from December 1969 to April 1972. Table 1 shows the diagnoses and the responses to adriamycin of these 127 patients. Twenty-seven patients had no followup EKG and were disqualified. No clinical evidence of cardiac disease was seen in any of these 27 patients during adriamycin therapy. The remaining 100 patients formed the basis of this report. There were 70 men and 30 women whose ages ranged from 15 to 77 years with a mean and median age of 46 and 50 years respectively.

EKG Changes

EKG abnormalities developed in 25 of 100 patients during adriamycin therapy. Such changes included nonspecific ST-T changes, prolongation of Q-T interval, atrial flutter and fibrillation with atrioventricular block, low R-wave voltage in limb leads, poor R-wave progression in chest leads, and combinations of any of the above, as shown in table 2. Eighty-six patients had normal EKGs before therapy. In this group various EKG changes developed in 19 patients (22%), and 67 patients (78%) retained their normal tracing. In contrast, of 14 patients with abnormal EKGs before therapy, six (42%) had further EKG abnormalities and eight (57%) retained their pre-treatment tracing.

Figure 1 shows the sample of various EKG changes resulting immediately after adriamycin

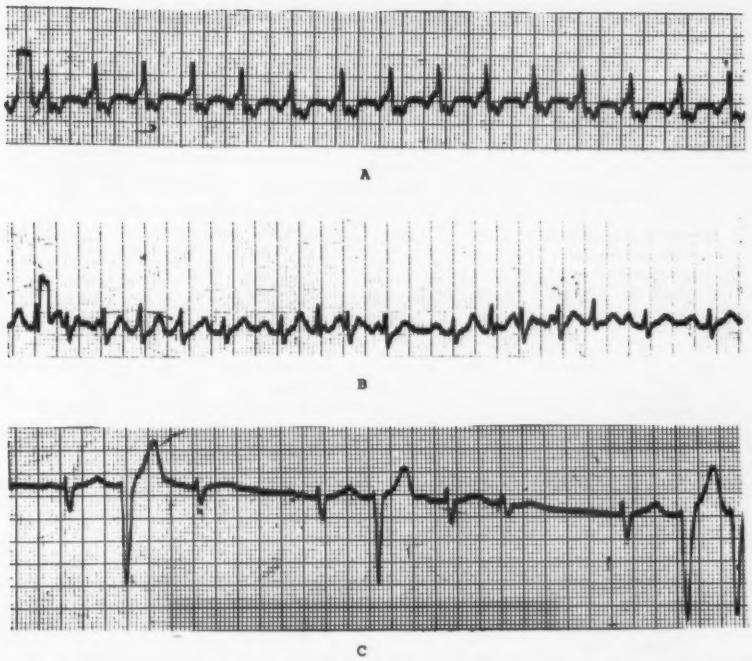

FIGURE 1.—Early EKG changes following adriamycin administration. (A) Atrial flutter with 2:1 atrioventricular block, (B) sinus rhythm with multifocal atrial premature beats, and (C) regular sinus rhythm with premature ventricular beats.

therapy. The premature atrial and ventricular contractions of figures 1B and 1C respectively usually reverted to normal spontaneously during the rest period without adriamycin, while the atrial flutter with atrioventricular block in figure 1A required aggressive antiarrhythmic medications in two patients and electroconversion in one patient before normal sinus rhythm was attained. Reinstitution of adriamycin in the last patient resulted in the reappearance of atrial flutter requiring discontinuation of adriamycin indefinitely. Figure 2 shows the low R-wave voltage, and figure 3 shows a sample of poor R-wave progression in chest leads resulting from adriamycin therapy.

CHF

CHF developed in nine of 100 patients in the phase I study of adriamycin. The cumulative dose of adriamycin given ranged from 510 to 1155 mg/m^2. The CHF occurred from 171 to 380 days after the start of therapy and from 28 to 65 days after the last dose of adriamycin. Table 3 shows the detailed clinical picture of the nine patients who had CHF, their cumulative dose of adriamycin and the autopsy findings. Except for patient No. 2 who died of cardiac arrest during thoracotomy, patient No. 7 who had superimposed massive pulmonary emboli, and patient No. 8 who had cerebral emboli, CHF in the remainder of the patients responded to conventional therapy. This treatment consisted of discontinuation of adriamycin, rapid digitalization with digoxin, oxygen inhalation, diuretics, bed rest, and low-salt diet. Death in three patients was due to cardiotoxicity, while death in six patients was due to tumor progression.

Autopsy Findings

Fifty-one of 71 patients (71%) who had died at the time of these analyses were given an autopsy. Seventeen of 51 (33%) revealed cardiac abnormalities by light microscopic examination. These abnormalities were either in the form of edema between myocardial fibers (fig 4); myocytolysis, disruption of nucleus, and sarcoplasm with vacuole formation (fig 5); myocardial fibrosis; or combinations of the above (these microscopic changes will be referred to as toxic cardiopathy).

Twelve of the 46 cases with no CHF given autopsies (26%) had toxic cardiopathy. None in this group had cardiac dilatation on gross examination. In contrast, four of five cases with CHF given autopsies (80%) showed biventricular dilatation. Toxic cardiopathy was seen in all five patients. Figure 6 shows the mural thrombus in the ventricle of patient No. 8, which was the source of his cerebral emboli.

TABLE 1.—Adriamycin activity in 127 adults with advanced cancer

Diagnosis	Total No. of patients	No. of patients with—* CR	PR	Percent response (CR + PR)
Bronchogenic carcinoma				
Adenocarcinoma	17		5	29
Large cell	6		1	16
Small cell	6		3	50
Epidermoid	2			0
	31		9	29
Sarcoma				
Osteogenic	15		6	40
Ewing's	3		1	33
Liposarcoma	2	1	1	100
Chondrosarcoma	2			0
Synovial cell	1		1	100
Hemangiosarcoma	1		1	100
Leiomyosarcoma	1		1	100
Pericytoma	1		1	100
	27	1	12	48
Testicular carcinoma				
Embryonal	3		2	66
Teratocarcinoma	1			0
Choriocarcinoma	1			0
Embryonal + seminoma	1		1	100
Embryonal + choriocarcinoma	1		1	100
Embryonal + teratocarcinoma	1		1	100
	8		5	62
Head and neck epidermoid carcinoma	7		3	63
Thyroid carcinoma	5	1	1	40
Breast carcinoma	5			0
Acute myelocytic leukemia	16	2		12
Acute lymphocytic leukemia	3	1		33
Reticulum cell sarcoma	11	4	3	63
Lymphosarcoma	3		1	33
Miscellaneous†	11			0
	127	9	34	33.8

*CR = complete remission; PR = partial remission.

†Colon adenocarcinoma (3 patients), unknown primary (3), malignant melanoma (2), cervical epidermoid carcinoma (1), renal carcinoma (1), and hepatoma (1).

TABLE 2.—EKG changes after adriamycin therapy

EKG changes	No. of patients
Normal or no changes	75
Abnormal EKG	
Arrhythmias	5
ST-T changes	5
Low R-wave voltage in limb leads	3
Low R-wave progression in chest leads	2
Combination of EKG changes	10
	25

TABLE 3.—Adriamycin cumulative dose and CHF in nine patients with cancer

Patient No.	Age/ sex	Diagnosis	Dose (mg/m^2)	EKG changes	No. of days from first to last dose	No. of days from last dose to CHF	Clinical cause of death	No. of days from start of therapy to death	Cardiac findings on autopsy
1	43/M	Osteosarcoma	510	Atrial flutter-fibrillation	168	38	Cancer	222	Myocardial fibrosis
2	50/M	Lung carcinoma	510	ST-T changes	143	28*	Cardiac arrest during thoracotomy	171	Dilated heart, interstitial edema
3	54/F	Lung carcinoma	645	Poor R-wave progression	170	49	Cancer	316	No autopsy
4	61/M	Lung carcinoma	720	Low R waves, poor R-wave progression	200	48	Cancer	335	Dilated heart, interstitial edema
5	74/M	Lung carcinoma	735	Poor R-wave progression	168	28	Cancer	343	No autopsy
6	58/M	Lung carcinoma	815	Atrial flutter-fibrillation, poor R-wave progression	176	34	Cancer	266	No autopsy
7	23/M	Synovial cell sarcoma	900	Low R waves, poor R-wave progression	256	9	Cardiogenic shock	264	Dilated heart, interstitial edema, pulmonary emboli
8	17/M	Osteosarcoma	1125	Normal	336	26*	Cardiogenic shock	377	Dilated heart, mural thrombus, interstitial edema, cerebral emboli
9	63/M	Lung carcinoma	1155	Low R waves, poor R-wave progression	315	65	Cancer	512	No autopsy

*CHF was not treated.

Table 4 shows the frequency of CHF and of toxic cardiopathy without CHF in 86 patients with normal EKG before adriamycin therapy. Only one of 67 patients (1.4%) who retained his normal EKG had CHF. Toxic cardiopathy in this group was seen in six of 33 autopsies (18%). In contrast, six of 19 patients (31%) who had an abnormal EKG following adriamycin therapy had CHF, and two patients in this group who had no CHF were found to have toxic cardiopathy.

Table 5 shows the correlation of adriamycin cardiotoxicity in 14 patients with abnormal EKGs before therapy. No CHF was seen in eight patients who had retained their tracing, despite three of six patients (50%) with abnormal cardiac findings on light microscopy. On the other hand, CHF was seen in two of six patients (33%) who had further changes in their EKG examinations.

Table 6 shows the relationship of adriamycin cumulative dose with CHF and with toxic cardiopathy without CHF. CHF developed in two of 90 patients (2.2%) receiving less than 600 mg/m^2 of adriamycin. In contrast, seven of ten patients (70%) receiving adriamycin therapy at doses over 600 mg/m^2 had CHF.

Phase II Study

Table 7 shows the relationship between cumulative doses of adriamycin and CHF in 150 children with various inoperable solid tumors. Only two patients had CHF after receiving adriamycin therapy at doses of 450 and 500 mg/m^2 respectively. Both patients had prior radiotherapy to the mediastinal area. None of the 15 patients receiving adriamycin at doses from 510 to 700 mg/m^2 had CHF. Twenty-one patients in this study had serial EKG examinations during adriamycin therapy. Three of these patients had EKG changes (nonspecific ST-T wave changes, first-degree atrioventricular block, and changes suggestive of left atrial enlargement) at doses of 360, 200, and 120 mg/m^2 respectively.

Adjuvant Study in Primary Osteosarcoma

All 44 patients in this study had received adriamycin at a dose less than 550 mg/m^2. None had CHF as shown in table 8. Twenty-three of 44 patients had received 540 mg/m^2 and had been without adriamycin therapy from 1 to 32.5 months with no evidence of any cardiac problems.

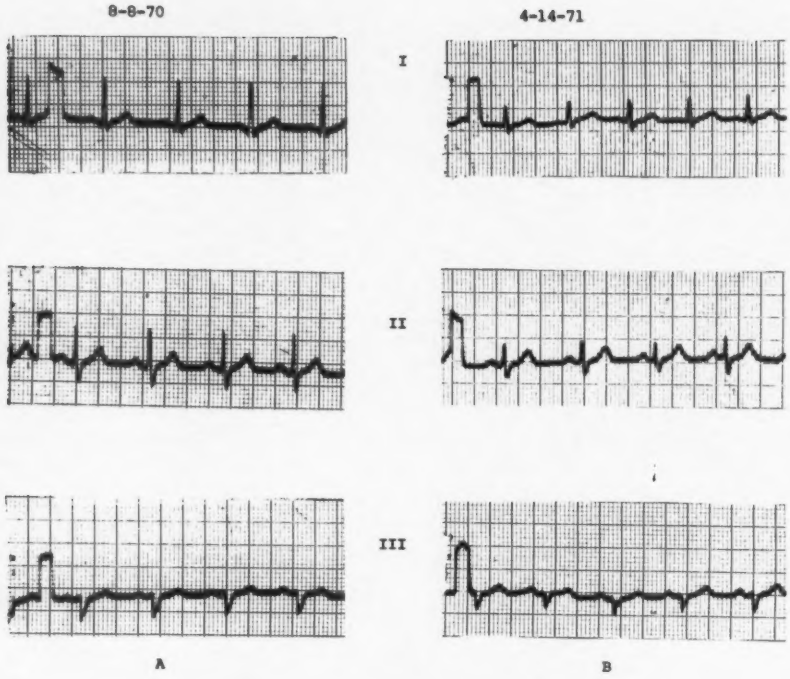

FIGURE 2.—EKG tracing before (A) and after (B) adriamycin therapy showing the low R-wave voltage after 540 mg/m².

DISCUSSION

The data presented demonstrate two types of adriamycin cardiotoxicity: *(a)* immediate or early, and *(b)* cumulative or late.

The immediate cardiotoxic effects of adriamycin occurred even after one course of therapy. These consisted of nonspecific EKG changes and toxic cardiopathy on microscopic examination. The EKG abnormalities included ST-T changes, prolongation of Q-T interval, premature atrial and ventricular contractions, and atrial flutter and fibrillation. These were all reversible, either spontaneously or by antiarrhythmic medications.

The cumulative cardiotoxic effects were nonspecific EKG changes and CHF with cardiac dilatation and toxic cardiopathy at autopsy. The EKG abnormalities were low R-wave voltage, poor R-wave progression in chest leads, and atrial flutter and fibrillation with atrioventricular block. The R-wave changes in the EKGs were nonreversible and they preceded the CHF seen in six patients in the phase I study.

Eleven of 294 patients in the series had CHF. The clinical manifestations of CHF from adriamycin in this study were: *(a)* sudden onset of shortness of breath, *(b)* neck vein distention, *(c)* gallop rhythm, *(d)* leg edema, and *(e)* hepatomegaly in some patients. Six of 11 patients did not have postmortem examination to confirm the cardiotoxicity or to exclude cardiac metastases. The absence of pre-existing clinical cardiac disease or cor pulmonale from metastases and CHF before tumor relapse or progression is evidence indirectly suggesting that adriamycin was the ultimate cause of CHF.

Lefrak et al (3) reported cardiomyopathy in none of 327 patients who received less than 500 mg/m², one of 366 patients (0.27%) who received less than 550 mg/m² (range, 45-545), and ten of 33 patients (30%) who received more than 550 mg/m² (range, 560-1040). In the present study CHF was seen in none of 224 patients who received less than 450 mg/m², four of 280 patients (1.4%) who received less than 600 mg/m² (range, 60-570), and seven of 13 patients (53%) who received more than 600 mg/m² (range, 615-1155). Both of these series demonstrate that the CHF resulting from adriamycin is dose related.

The other factor which influences the occurrence of CHF is the presence or absence of abnormal EKG before the start of adriamycin therapy. While CHF was found in seven of 86 patients (8%) with a prior normal tracing, CHF was noted in two of 14 patients (14%) in the group with abnormal EKGs before

TABLE 4.—Adriamycin-induced cardiotoxicity in 86 patients with normal EKGs

Followup EKG	No. of patients	No. of patients with CHF	No. of autopsies/No. of deaths	No. of cases of toxic cardiopathy without CHF/No. of autopsies
Normal	67	1	33/49	6/33
Abnormal				
Arrhythmias	5	2	2/4	
ST-T changes	4		4/4	2/4
Low R-wave voltage	3			
Low R-wave progression	2	1		
Combination of changes	5	3	2/3	2/8
	19	6	8/11	
Totals	100	7	41/60	8/41

TABLE 5.—Adriamycin-induced cardiotoxicity in 14 patients with abnormal EKGs

Followup EKG	No. of patients	No. of patients with CHF	No. of autopsies/ No. of deaths	No. of cases of toxic cardiopathy without CHF/ No. of autopsies
No change	8	0	6/7	3/6
ST-T changes	1			
Combination of changes	5	2	4/4	1/4
Total	14	2	10/11	4/10

TABLE 6.—CHF and toxic cardiopathy in relation to adriamycin cumulative dose

Dose (mg/m^2)	No. of patients	No. of patients with CHF	No. of patients with toxic cardiopathy*
<450	75	0	11
451-500	3	0	1
501-550	11	2	2
551-600	1	0	
>600†	10	7	3
Totals	100	9	17

*Twelve of 46 patients with toxic cardiopathy in the absence of CHF and 5 of 5 patients with toxic cardiopathy.

†Adriamycin cumulative doses of 615, 715, 720, 735, 810, 815, 840, 900, 1125, and 1155 mg/m^2.

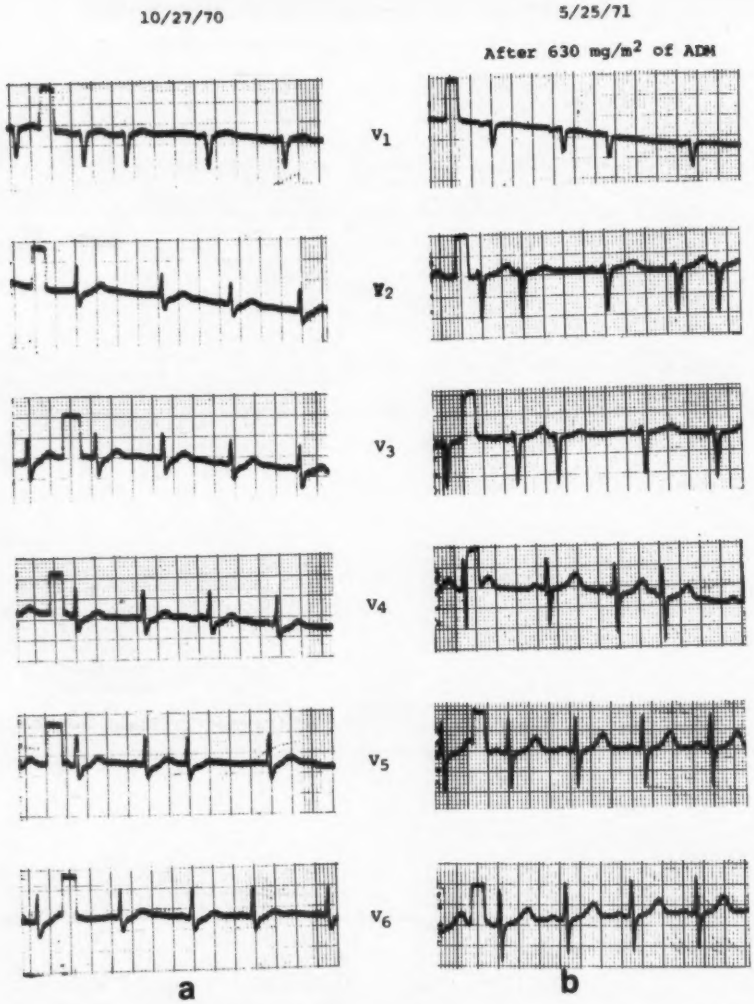

FIGURE 3.—Before (a) and after (b) EKG tracing of a 74-year-old man, showing the poor progression of R waves after 630 mg/m² of adriamycin (ADM).

TABLE 7.—Adriamycin cumulative dose and CHF in 150 children with cancer

Dose (mg/m²)	No. of patients	No. of patients with CHF
<450	129	0
451-500	6	2
501-550	7	0
551-600	5	0
>600*	3	0
Total	150	2

*Adriamycin cumulative doses of 615, 680, and 700 mg/m².

TABLE 8.—Adriamycin dose and CHF in primary osteosarcoma

Dose (mg/m²)	No. of patients	No. of patients with CHF
<450	20	0
451-500	1	0
501-550	23	0
Totals	44	0

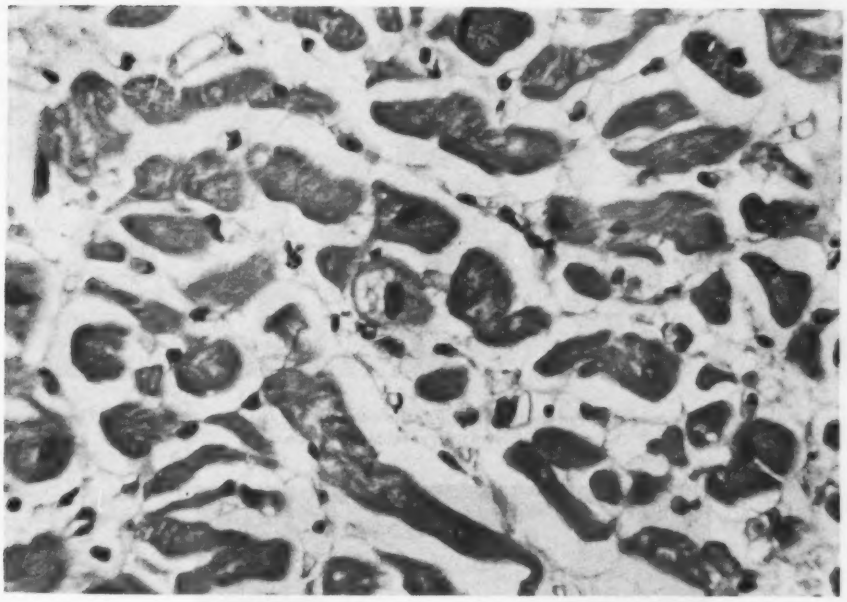

FIGURE 4.—Interstitial edema and generation of the myocardial fibers.

treatment. Patients with pre-treatment abnormal EKGs require particularly careful cardiac monitoring during adriamycin therapy.

Lefrak et al (3) reported the refractoriness of adriamycin-induced CHF in eight of ten patients. Their result is in sharp contrast to the present study where six of seven patients with CHF responded to rapid digitalization, diuretics, oxygen inhalation, bed rest, and low-salt diet. These six patients subsequently survived to die of cancer, not of cardiac disease (table 3). Gilladoga et al (5) also reported that adriamycin-induced CHF in children responded to the above treatment.

The immediate and cumulative cardiotoxicity of adriamycin in this series correlates well with the study of Rinehart et al (6). Their group measured ventricular function by means of the ratio of pre-ejection period and left ventricular ejection time (PEP/LVET) during adriamycin treatment. They demonstrated a transient worsening of left ventricular function in four patients whose cumulative doses were less than 200 mg/m². The PEP/LVET ratio showed a progressive, gradual increase as more adriamycin was given and usually became abnormal at cumulative doses greater than 300 mg/m². Three of their four patients with adriamycin-induced left ventricular dysfunction that persisted more than 1 month after therapy had significant improvement in PEP/LVET with digoxin administration.

The mechanism of adriamycin cardiotoxicity, like that of daunorubicin (8),[10] is still unknown. Mhatre et al (9) in a series of experiments on isolated dog heart preparations perfused with blood showed that adriamycin and daunorubicin produced an immediate and sustained increase in pump perfusion pressure. It is possible that repeated injection of either of these agents in man will ultimately cause CHF through the gradual and sustained pump pressure. The accumulation of the aglycone metabolite of adriamycin may be the agent responsible for the myocardial dysfunction (9,10). However, the ultimate mechanism by which it causes muscle damage and heart failure remains to be determined.

The present study demonstrates that adriamycin is an active agent against various neoplasms. Its

[10]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

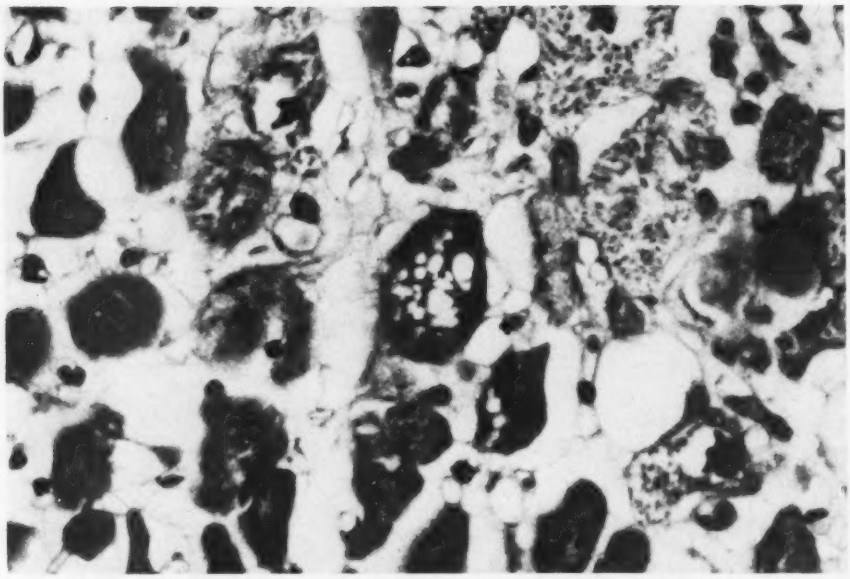

FIGURE 5.—Light microscopy of the myocardium showing myocytolysis, disruption of nucleus, and sarcoplasm with vacuole formation.

FIGURE 6.—Presence of cardiac dilatation and mural thrombus of the ventricle on gross examination in patient No. 8.

administration, however, must be limited to its cardiotoxic threshold of less than 600 mg/m^2 (cumulative dose). The CHF induced by adriamycin is treatable. Therefore, vigorous support to reverse this incapacitating complication deserves a trial.

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Rational Bases for Adriamycin (NSC-123127) Combination Therapy[1,2]

Thomas C. Hall[3,4]

Since a number of anticancer agents induce chemical responses at rates that are reproducible, but less than 50%, and since pharmacologic studies show that many drugs have different modes of action, combination drug therapy has been used in some patients. The initial success of combination therapy in childhood leukemia has been followed by improved combination therapy for lymphoma, adult leukemia, and breast cancer. As new and effective agents become available, it becomes necessary to evaluate their roles in combination therapy. And as more of these agents are added to the active list, it is more difficult to demonstrate useful combinations by empirical trials because it is harder to obtain enough patients for the enlarging numbers of possible combinations. Hence, a rational basis is needed for the selection of the more successful combinations. This is especially important when a drug has unique characteristics such as the long plasma half-life and cardiotoxicity of adriamycin. Realistically though, knowledge of the pharmacology of most drugs is very limited; the likelihood is also great that some strictly empirical combinations will be tried and found effective.

GENERAL CONSIDERATIONS

There are a number of variables in the design of any drug combination:

a. number of drugs;
b. classes of action at the cellular level;
c. differences in handling by the host;
d. simultaneous or sequential administration schedules; and
e. differential toxic effects upon normal tissue.

In addition, there are several different goals of combination therapy:

a. to increase antitumor effects;
b. to inhibit the development of drug resistance;
c. to exploit possible drug interactions; and
d. to spread host toxicity among several organs and decrease the maximum toxic effect on host systems.

With adriamycin, the possibility exists that combinations would permit remission induction at lower single adriamycin doses, and thus by decreasing the average dose, one could prolong the time taken to reach the cumulative cardiotoxic adriamycin dose range.

TEMPORAL CONSIDERATIONS

The order in which combination therapies are given is critical in determining response. The drugs involved in combination therapy can be given simultaneously or separated by time intervals that may vary from ones calculated in minutes to ones as long as weeks. The administration time for each drug may also vary from short boluses to continuous infusions lasting many days. Which drug comes first is also of considerable importance. Adriamycin, which appears to create its major effect by intercalating with preformed DNA residues rather than by interfering with the time-consuming synthesis of new DNA, has an apparent advantage when it is given as a followup drug, since it does not have a mode of action that interferes with a DNA synthetic process. Furthermore, since adriamycin has a prolonged plasma half-life, there is little reason to give it by prolonged infusions or even at intervals that are less than 2 or 3 days. Thus, adriamycin might best be used as a "single-bolus" drug given after other shorter-acting drugs have brought an increased number of tumor cells into a stage in the mitotic cycle at which they contained large amounts of adriamycin-sensitive DNA.

Sequential combination therapies are based upon the assumption that, at best, some of the host or target cells are proceeding through the mitotic cycle and a drug could alter the rate in order to increase tumor cell S-phase synchronization, or block normal cell progression, or both. Simultaneous drug combinations are designed to exploit the heterogeneity of tissue, often due to presumed polyclonal tumor composition, with different phenotypes exhibiting different drug sensitivities. Alternatively, and particularly in the case of slow-growing solid tumors, combination chemotherapy is designed to hit presumably monoclonal tumor cells, one drug for S phase and one for G_1. In the case of slow-growing tumors, adriamycin might be reasonably included in such a simultaneous combination as a drug effective by direct cytotoxicity against the G_1 cells. In addition, adriamycin could be given at or after the time of administration of radiation or mustard therapy, both of which create structural lesions in DNA. This is because the intercalating action of adriamycin might well cause amplification of such induced lesions, or interfere with normal DNA repair processes, or decrease the stability of the final DNA.

[1]Supported in part by grant No. CA-14089 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[2]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

[3]Advanced Therapeutics Program, Los Angeles County/University of Southern California (LAC/USC) Cancer Center. Recipient of American Cancer Society Research Professorship PRP-47.

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MOLECULAR ASPECTS

When several drugs are given in combination but as separate physical entities, the rate and dose schedule of each can be varied independently until the optimal combination is found. In a number of instances a single molecular species has been synthesized from two drugs. In this case the proportions of the component drugs and the schedules and rates of administration become fixed by the properties of the new molecule, with a concomitant loss of therapeutic versatility. In some instances, such as with the drug estradiol mustard, the resulting molecule may be less effective than the best dose combination of its components when given separately (1). It may possibly even lose critical therapeutic characteristics such as binding to a specific receptor. However, the selection of proper components may result in other desirable qualities such as transport of the molecule into specific tumor cells. Such "site-directed" considerations underlie the synthesis of combinations of mustards and amino acid agents by Soviet investigators. In the case of daunorubicin and now adriamycin, this carrier concept led to the preparations of complexes with DNA in the hope that the macromolecule would carry the drug into the tumor cells by pinocytosis, which was more apt to be active in such cells (2). It was also hoped that after such ingestion the active lysosomal hydrolytic enzymes of the tumor cell would then release free active drug within the target tumor cells. Preliminary data from Belgium and Norway suggest that such daunorubicin and adriamycin complexes may indeed be as effective and less cardiotoxic than the free drug alone. These data need to be confirmed by others. Even if they are proved to be effective, the rationale cannot be taken as documented until studies demonstrate actual increased uptake, differential distribution of lysosomes in cardiac versus tumor tissue, and definite lysosomal hydrolysis of the DNA drug complex.

One of the great pharmacologic gap areas is our inability to extrapolate from molecular modes of action to useful clinical therapeutic situations. Hence, we know that on the molecular level adriamycin intercalates with DNA. On the biochemical level, we know that some disturbance of DNA to RNA transcription occurs. But these actions are insufficient for us to predict that some tumors, such as lymphoma, will be sensitive where others, such as colon cancers, are not. We also do not know the molecular basis for the resistance to drug seen in at least 50% of all patients with apparently identical lymphomas. There is a need for more support of biochemical work in order to exploit optimally at the cellular level the knowledge that we have accumulated at the molecular level.

At the cellular level, there is good evidence that transport into tumor cells may be critical for the anthracyclines and that drug-induced resistance to any large heterocyclic compounds, including cyclophosphamide, vincristine, daunorubicin, and camptothecin, may carry collateral resistance to one or more of the others in this group (3).

PHARMACOLOGIC GOALS OF COMBINATION THERAPY

Sequential Metabolic Inhibition of DNA Synthesis

This approach was first described by Potter and has been thought of primarily in relation to known segmented and inhibitable steps in a critical biochemical pathway (4). As has been discussed, the mode of action of adriamycin does not seem to involve inhibition of such a definite step in DNA synthesis, and presumably this is not an easily exploitable concept for adriamycin combinations. The same is true for the complementary blockade (described by Elion) by two drugs of precursors to a critical tumor product (5). These, plus other combination rationales such as the complementary inhibition of Sartorelli (6), the metabolic sensitization as described by Hall (7), and the metabolic activation and conditioning of Bloch (8), all involve drug action at points during DNA synthesis, and since there is little evidence that adriamycin acts preferentially at such loci, there is not much reason to suppose that adriamycin would form an effective combination directed toward the "S" processes within the tumor cell. However, since most tumors are not synchronized in cellular replication, the possibility exists that adriamycin combined with a mitotoxic DNA antimetabolite might find each drug effective against different cells within the target tumor mass. Presumably this would involve a cytotoxic action of adriamycin against early G_1 and S cells. Specific combinations would be built upon previous demonstrations of independent actions of each drug against the tumor type involved. From what we know of the effects of single antimetabolites and of adriamycin, such a combination could add to the effective treatment of leukemias and possibly gastric cancer. However, mitotoxic drugs comprise only a part of the several types of currently available antitumor drugs. The other drug types act primarily on cells which are not in mitosis and it would probably be beneficial to consider possible combinations between adriamycin and some of these.

Oncoprival Therapies

Oncoprival therapies deprive a tumor cell of small amounts of normal critical growth substances which are required for cell replication (9). This would include castration of the premenopausal breast cancer patient to remove endogenous low levels of estrogens. The targeted cells in the tumor are those which are dividing slowly and which represent a different group from those against which adriamycin might be effective. Thus adriamycin might provide a spe-

cific benefit when added to oophorectomy, adrenalectomy, or orchiectomy.

Ontoductive Therapy

Physiologic regulators, when given at many-times-normal physiologic concentrations, act upon the partially differentiated phenotype of the tumor cell to promote its differentiation toward a nondividing end-stage parenchymal cell. This is also a therapeutic action which moves cells out of S and M into prolonged G_1 phase, at which time the DNA might still be sensitive to adriamycin. High-dose estrogen therapy for breast cancer, progesterone therapy for endometrial cancer, or vitamin A therapy for epitheliomas and chondrosarcomas might thus provide a good basis for adriamycin combinations. The same is true for *ontatrophic therapy* of secondary sex tumors such as prostate and breast with the "contralateral" steroids in high doses, such as estrogen and androgen respectively. *Histotoxic* therapy, which attempts to destroy one normal tissue selectively within the body and any tumor derived from the normal tissue, requires a very high degree of drug toxicity for a specific normal tissue. This is seen in L-asparaginase destruction and corticoid destruction of normal and malignant lymphocytes, mitotane destruction of normal and malignant adrenal cortical cells, and streptozotocin destruction of normal and malignant pancreatic islet cells. Adriamycin's only selective tissue toxicity seems to be to the heart and thus it might be considered as a possible therapy for the rare tumor, cardiac rhabdomyosarcoma. In this case, a combination might be sought that had *negative* synergism, ie, it would block the effect of adriamycin on the normal heart. Alternatively, local therapy that would have an additive effect (such as local radiation therapy) might be considered.

The possibility that negative synergism by combination therapy would give a net increase in therapeutic ratio has not been sufficiently discussed. In the case of adriamycin, it seems likely that cardiac toxicity is due to intercalation in DNA and blocking of effective transcriptions of DNA messages into critically required RNA species in this rapidly metabolizing but nondividing tissue. One could consider pretreatment of the host with nontoxic analogs of adriamycin which might induce detoxifying metabolic patterns in normal heart tissue. Tumors do not usually show substrate induction of enzymes and thus can not build up the same protective catabolic machinery. Later treatment with active adriamycin could then intoxicate the tumor selectively. Another approach might be to pretreat the host myocardium with drugs that intercalate with cardiac DNA but are in themselves nontoxic. Such drugs might include antimalarials, phenothiazines, or tetracyclines.

CLINICAL CONSIDERATIONS

Applying these pharmacologic principles should reveal several points.

1. Adriamycin plus antimetabolites would:
   a. not be synergistic,
   b. be additive in only those tumors in which independent antitumor activity had already been shown for each drug, and
   c. be largely restricted to the leukemias, and breast and gastric tumors.
2. Adriamycin in combination would be given optimally in boluses with or after other drugs.
3. Adriamycin given before radiation therapy might sensitize tumors to local radiation therapy.
4. The combination of adriamycin with drugs active against G_1 cells, such as cyclophosphamide, vincristine, and steroids, might be particularly effective.
5. Cells developing resistance to antitumor bulky heterocyclic molecules might often develop collateral resistance to adriamycin, and hence adriamycin combinations in such resistant patients might be particularly toxic yet ineffective.
6. Actual reference to the combinations either reported in the literature, discussed at the Adriamycin Symposium in December 1974 in Washington, DC, or under way at this time tends to confirm these conclusions.

The following generalizations seem valid conclusions to be drawn from clinical reports: *(a)* the simultaneous use of cyclophosphamide and adriamycin in combination seems to have additive antitumor effect but, unfortunately, possibly additive cardiotoxicity; *(b)* anti-DNA antimetabolites plus adriamycin seem additive in acute leukemia; and *(c)* adriamycin combinations increase the response rates of tumors known to be sensitive to the individual drugs, but do not appear to render insensitive tumors sensitive.

A number of potential combinations, as yet untried, are suggested by the following considerations:

a. The use of adriamycin with hormone therapy and 5-fluorouracil in breast, prostate, and endometrial cancer seems logical.
b. A trial of adriamycin and 5-fluorouracil in gastric or bladder cancer seems worthwhile.
c. Adriamycin plus cyclophosphamide plus progestins might be worthwhile in endometrial and prostate cancer.
d. Adriamycin, vincristine, cyclophosphamide, prednisone, and bleomycin might be effective in lymphoma or oat-cell cancer.
e. Adriamycin and hypervitaminosis A seem indicated in chondrosarcoma and epidermoid carcinoma.
f. Since they may share a common transport block in resistance, the large heterocycles may best be given simultaneously, and not after resistance to any one of them has developed.
g. Adriamycin pretreatment may sensitize to localized radiation, or since it is fluorescent, to localized ultraviolet or electron radiation, in those superficial tumors in which it can be shown to be taken up.

h. Cardiotoxicity might be decreased by pretreatment with catabolic enzyme inducers or nontoxic intercalators with cardiac DNA.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
L-Asparaginase: NSC-109229; CAS reg. No. 9015-68-3
Bleomycin: NSC-125066
Camptothecin: NSC-100880; CAS reg. No. 25387-67-1
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6; daunomycin
Estradiol mustard: NSC-112259; CAS reg. No. 22966-79-6; estra-1,-3,5(10)-triene-3,17β-diol, bis[[p-[bis(2-chloroethyl)amino]phenyl]-acetate]
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8
Mitotane: NSC-38721; CAS reg. No. 53-19-0; ethane, 1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)-; o,p'-DDD
Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-
Streptozotocin: NSC-85998
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Adriamycin (NSC-123127) Studies at the Istituto Nazionale Tumori, Milan[1]

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SUMMARY

This paper reports the therapeutic results and the toxic manifestations of adriamycin administered as a single agent and in combination with conventional drugs in the treatment of various types of neoplastic disease in adults and children. The studies indicate that this new drug appears to be effective in a large variety of tumors and lacks cross resistance to practically all standard agents. Objective response was not observed in patients with chondrosarcoma or clear cell carcinoma of the kidney. In a number of controlled studies, all combinations that included adriamycin proved, in terms of remission rate, to be therapeutically superior or at least as effective as those regimens that included conventional drugs. The intermittent dose schedules were found to be well tolerated in terms of severe hematosuppression and stomatitis. The overall response rate of the present series did not appear to be dependent on the dose schedule. The highest incidence of complete response, however, occurred after the high-dose intermittent schedules. Cardiomyopathy was observed in a total of 11 of 500 patients (2.2%) who received adriamycin alone, occurring after a median dose of 560 mg/m^2. Heart failure was fatal in four of eleven patients. This dose-limiting side effect occurred in only two patients after receiving doses of 412 and 600 mg/m^2, respectively, when adriamycin was administered in combination.

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Adriamycin became available for treatment in man during the summer of 1968. The initial phase I and II studies were carried out by the medical oncology group at the Istituto Nazionale Tumori of Milan from September 1968 to June 1970. During this period of time, some of the most important properties of this new antibiotic were observed, namely the general toxic signs, the prompt therapeutic effect after low doses, the wide range of responsive tumors, and the lack of cross resistance to many standard drugs (1,2). The problem of cardiac toxicity also emerged during the initial evaluation (2), although, from the initial series of patients, neither a risk dose nor the clinical criteria for drug-induced cardiomyopathy could be defined. Since 1969, adriamycin has been extensively evaluated, especially in the United States where the usefulness of loading intermittent schedules was observed (3,4) and the modern concepts of cardiomyopathy were developed (5,6). Furthermore, the drug was found to be active in some otherwise poorly responsive neoplasms such as carcinoma of the urinary bladder (5), osteogenic sarcoma (7), and thyroid carcinoma (8,9). Since 1970, our Institute and gradually other centers throughout the world have introduced adriamycin into several drug combinations (10-19), and, more recently, adriamycin has been used in combined treatment modalities (20-24) for susceptible neoplastic diseases.

The aim of the present report is to summarize and update our experience with this new antibiotic administered as a single agent and to outline most of the recent and ongoing trials with adriamycin in combination regimens. The results of the combination usage of adriamycin in malignant lymphomas are reported in detail separately (25).

PATIENTS AND METHODS

Patient Selection

A total of 500 patients (433 adults and 67 children) were considered evaluable for both therapeutic and toxic effects of adriamycin as a single agent. In contrast to our previous reports (26,27), some of the patients treated during the phase I study were deleted from the present analysis because they had received insufficient therapy to allow an adequate evaluation of drug response in terms of therapeutic effects. All patients were thoroughly reevaluated according to histopathology, clinical parameters,

[1]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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incidence of toxicity, and type of response. A total of 208 of 500 patients (42%) had previously received one or more standard drugs. The performance status ranged from 40 to 80.

Since June 1970, adriamycin has been gradually introduced into several multiple-drug treatment programs and, more recently, into combined treatment modalities for various types of susceptible neoplastic disease. The large majority of patients receiving combination chemotherapy had not previously received conventional drugs. Their performance status ranged from 60 to 90.

Drug Administration

During phase I and II studies, adriamycin was administered intravenously (iv) in six different dose schedules as previously reported (26,27). As seen in table 1, there was a gradual tendency toward the use of intermittent schedules. Schedules E and F have been employed since September 1970, after the initial experience conducted at the M.D. Anderson Hospital and Tumor Institute (Houston, Tex) (3). With the exception of those patients treated during the phase I study, the large majority of patients were treated in the outpatient clinic. Adriamycin was usually administered as long as it was evident that the disease was not progressing.

In the design of multiple-drug protocols that included adriamycin, the strategic approach was primarily that of developing two independent, effective, and theoretically noncross-resistant combinations to be used sequentially in future treatment studies. Crossover was, in fact, carried out in the presence of progression or relapse. The experience achieved through the use of intermittent dose schedules was applied when adriamycin was administered in combination with other drugs. Treatment protocols were designed to avoid a total dose in excess of 550-600 mg/m^2 during the induction phase. When approaching this risk dose, patients were crossed over to the other combination. Practically all patients were managed on an outpatient basis. When myelosuppression occurred, a temporary dose-attenuation schedule was employed. For patients given schedules E and F, the dose adjustment was made on the basis of leukocyte and platelet counts determined on the same day the drug was administered. There were only a few patients in whom the nadir of the leukocyte and platelet counts was systematically determined and the dose modified accordingly. This fact is important when the incidence of myelosuppression is analyzed.

Evaluation of Response

All patients who were entered into the different studies had histologically proven diagnoses of neoplastic disease. For patients biopsied elsewhere, slides and/or paraffin blocks were reviewed by members of the Division of Pathology of our Institute. Whenever possible, a histopathologic diagnosis of metastatic disease was also obtained.

All patients included in the present analysis had one or more measurable parameters for drug response as determined through physical and various radiologic examinations carried out according to histopathologic diagnosis and clinical presentation. During the drug evaluation period, physical examination, biochemical tests, ECGs, X-ray films, and scans were periodically repeated.

The criteria for evaluating the degree of drug response were as follows:

Complete response (CR).—Disappearance of all signs and symptoms of disease, including recalcification of osteolytic metastases and a return to normal levels of abnormal biochemical tests for at least 1 month.

Partial response (PR).—A 50% or greater decrease in the longest perpendicular tumor diameters for a minimum of 1 month.

Objective improvement.—A 25%-50% decrease in the longest perpendicular tumor diameters for a minimum of 1 month.

No response or progression.—Mixed-type regressions were always considered as no response. Pro-

TABLE 1.—Adriamycin iv dose schedules employed at the Istituto Nazionale Tumori at Milan

Regimen	Dose	Schedule
A	0.4,0.6,0.8 mg/kg (15,23,30 mg/m^2)	Daily × 4, rest 3 days, then once or twice weekly
B	Same	Every other day × 4-5, then once or twice weekly
C	Same	Daily × 3, rest 4 days, daily × 4, rest 4 days, then once or twice weekly
D	Same	Same as C except rest 7 days
E	20-25 mg/m^2	Daily × 3, repeat q3wks
F	60-75 mg/m^2	Single dose repeated q3wks

gression was defined as an increase of at least 25% in the tumor measurements or the appearance of new lesions even in the presence of regression elsewhere.

RESULTS

Toxicity of Single-Agent Therapy

Table 2 reports the principal toxic manifestations. As described in previous publications (26,27), loss of hair to complete alopecia, oral mucositis, and bone marrow suppression represent the major short-term toxic manifestations. Various degrees of nausea and vomiting occurred in the large majority of patients. In about 3% of the patients, hyperpigmentation of nail beds and dermal creases and rash, including urticaria around the vein selected for drug injection, were observed. The general toxic effects were reversible. Irreversible marrow aplasia occurred in only 11 of 155 patients (7%) in the initial series (2). In relation to the six schedules employed, no significant difference was seen in the incidence of alopecia. A progressive decrease, however, in the incidence and degree of stomatitis was observed by prolonging the interval between courses. The incidence of oral ulcerations was practically nil with schedules E and F, while redness of oral mucosa was observed in 46% and 23%, respectively. The same applies to myelosuppression, but this observation was limited to the group treated with schedule F. Thrombocytopenia occurred in about 10% of the patients. Bone marrow suppression was usually more prominent in elderly patients and in those who had previously received intensive radiotherapy and/or chemotherapy.

The delayed type of toxicity is represented by cardiomyopathy (28,29), which is presently recognized as the major factor limiting the long-term use of adriamycin in the present series, the clinical criteria used by Lefrak et al (6) were adopted to define adriamycin-induced cardiomyopathy. Cardiomyopathy occurred in a total of 11 of 500 patients (2.2%). In patients treated with the first four schedules, cardiac failure was detected in three of 256 patients (1.1%) after a median total dose of 210 mg/m^2 (range, 205-900 mg/m^2) as compared to eight of 244 patients (3.2%) receiving the high-dose intermittent schedules E and F after a median total dose of 570 mg/m^2 (range, 310-1155 mg/m^2).

As observed by practically all investigators (28), the incidence of cardiomyopathy is directly proportional to the total dose administered and rises sharply after a 600-mg/m^2 dose (table 3). The main clinical findings in patients with cardiomyopathy are summarized in table 4. First of all, in more than one half of the patients cardiac toxicity was not fatal, and in seven of 11 patients the heart failure gradually improved with supportive therapy. In the present series, the median total toxic dose and the median age were lower in patients with fatal cardiomyopathy as compared to those with nonfatal cardiomyopathy. Cardiac toxicity occurred in two children, and one of them (age 3 years) died of congestive heart failure. The median interval between the last dose of adriamycin and the onset of symptoms and signs was shorter in the patients who died of heart failure as compared to those who survived. Furthermore, in patients with fatal toxicity, the interval between the onset of symptoms and death was 2-4 days. Congestive failure occurred in most patients. No patient had received radiotherapy to the mediastinum and/or the lungs.

Besides pump failure, about 15% of the patients showed, during the course of treatment, aspecific, dose-independent, and reversible electrocardiographic changes. In most patients the ECG abnormalities were compatible with myocardial ischemia. As shown in a previous publication (2), these abnormalities did not predict, per se, the subsequent occurrence of life-threatening heart failure.

Toxicity of Combination Chemotherapy

This topic has been described in detail in separate reports (12-14,30,31). Briefly, the various general side effects were transient and their incidence appeared acceptable. Despite temporary episodes of myelosuppression, a minimum of 80% of the optimal dose for each drug, including adriamycin, was administered during the first six cycles of therapy. Definite cardiomyopathy was observed in only one patient. This patient, a 47-year-old woman with advanced breast cancer, had received eight consecutive cycles of adriamycin plus vincristine at a full dose level (total dose 600 mg/m^2) due to lack of bone marrow depression between courses. After the crossover to cyclophosphamide, methotrexate, and

TABLE 2.—Toxicity related to the six dose schedules employed

Side effects	Percent of patients experiencing toxicity on dose schedule— A (25)*	B (17)	C (97)	D (117)	E (115)	F (129)
Alopecia	80	81	87	83	85	90
Stomatitis	68	69	78	47	46	23
Myelosuppression†	72	63	86	63	75	45
Cardiomyopathy	4	0	1	0.9	4.3	2.3
None	0	6	1	5	7	12

*No. of patients in each group.
†Leukocytes <4000 cells/mm^3; platelets <150,000 platelets/mm^3.

TABLE 3.—Incidence of cardiomyopathy in relation to total dose

Total dose (mg/m²)	No. of patients	No. of patients experiencing cardiomyopathy (dose schedule used)		Incidence (%)	
		Fatal	Nonfatal		
<200	267			0	
201-300	124	2 (A,C)		1.6	1.9
301-400	32	1 (E)		3.1	
401-500	31		2 (E,F)	6.4	7.2
501-600	24	1 (F)	1 (F)	8.3	
601-700	11		1 (E)	9	18.1
701-800	0				
>800	11	—	3 (D,E,E)	27	
Totals	500	4	7	2.2	

TABLE 4.—Characteristics of patients with cardiomyopathy

	Cardiomyopathy		
	Fatal (4 patients)	Nonfatal (7 patients)	Total (11 patients)
Median total dose (mg/m²)	250	600	560
Range	200-575	409-1155	200-1155
Median age (yrs)	18	39	39
Range	3-54	10-65	3-65
Median interval from last dose to symptoms (days)	2	30	20
Range	1-75	13-60	1-75
Median interval from onset of symptoms to death (days)	3		
Range	2-4		
No. of patients with cardiac failure			
Forward	2	1	3
Congestive	2	6	8
No. of patients with abnormal ECG*			
Baseline	2	2	4
During adriamycin	3	5	8

*Tachycardia and/or flattened or inverted T-wave and/or premature ventricular contraction.

5-fluorouracil (5-FU), an irreversible congestive heart failure developed and she died at home 45 days after receiving the last dose of adriamycin. However, in another patient with cancer of the uterine cervix, reversible symptoms and signs compatible with cardiac toxicity occurred after a total dose of 412 mg/m².

Response to Single-Agent Therapy

The wide range of neoplastic diseases found to be highly responsive to adriamycin is listed in table 5. In myeloproliferative disorders and in multiple myeloma, the number of patients is too small to provide definite conclusions. In lymphoproliferative disorders, adriamycin appears particularly effective in histiocytic lymphoma. In the group of testicular tumors, PR was observed in three of six patients with seminomas and in five of 16 patients with embryonal carcinomas, while a CR which lasted for 18+ months was observed in one patient with choriocarcinoma. In patients with breast cancer, CR or PR was seen in three of 14 patients with dominant lesions in viscera as compared to four of 13 patients with dominant lesions in soft tissues. One patient with diffuse osteolytic lesions as well as soft tissue

TABLE 5.—Neoplastic diseases highly responsive to adriamycin

Disease	No. of patients	CR + PR (%)	No. of patients with CR	Response rate (%)	Median duration of response (mos)
Chronic myelogenous leukemia	7	86	2	86	2
Chronic lymphocytic leukemia	10	70	1	70	2
Acute lymphoblastic leukemia	9	66	4	66	1
Leukosarcoma	4	50	1	50	1
Multiple myeloma	8	63	0	63	2
Histiocytic lymphoma	21	67	2	81	2
Lymphocytic lymphoma	15	33	2	80	1.5
Hodgkin's disease	27	33	1	78	5
Thyroid carcinoma	11	45	2	55	2
Testicular tumor	23	39	1	57	4
Breast carcinoma	28	29	2	39	4
Ewing's sarcoma	18	39	1	56	2
Wilms' tumor	11	55	3	73	4

TABLE 6.—Neoplastic diseases moderately responsive to adriamycin

Disease	No. of patients	CR + PR (%)	No. of patients with CR	Response rate (%)	Median duration of response (mos)
Bladder carcinoma	6	33	0	33	4
Neuroblastoma	16	38	1	63	4.5
Nasopharyngeal carcinoma	16	31	2	50	8
Ovarian carcinoma	18	28	2	44	2.5
Soft tissue sarcoma	50	22	0	62	3
Mesothelioma	8	25	2	25	15.5
Bronchogenic carcinoma	49	14	2	45	4

and visceral metastases achieved CR with complete recalcification of bone involvement for 34 months. An encouraging response rate was also obtained in childhood neoplasias such as Wilms' tumor and Ewing's sarcoma. In this series of patients, the median duration of response was usually short; with only a few exceptions, response never lasted more than 6 months.

Table 6 presents the group of patients with neoplasms in which adriamycin exerted a moderate, though interesting, therapeutic activity. As in the previous group, the number of patients achieving CR is small. Again, however, the wide range of responsive tumors is remarkable. It is noteworthy that regressions were observed in patients with transitional carcinomas of the urinary bladder (two of six), liposarcomas (three of five), and mesotheliomas (two CR of eight). Beneficial response to adriamycin in patients with bronchogenic carcinoma remains low. By cell type, CR or PR occurred in one of 12 patients with epidermoid carcinoma, two of seven with small cell carcinoma (both patients had CR), three of ten with large cell carcinoma, none of nine with adenocarcinoma, and one of 11 with undetermined histology.

Table 7 lists the neoplastic diseases thought to be poorly responsive or totally unresponsive to adriamycin. In gastrointestinal tumors, regressions were observed in three of nine patients with gastric carcinoma (PR, two; objective improvement, one) and in four of six patients with colorectal carcinoma (PR, two; objective improvement, two). No response was seen in one child with hepatoblastoma and objective improvement was attained by one of two children with hepatocellular carcinoma. In patients with malignant melanoma and head and neck epidermoid carcinomas, the incidence of CR plus PR was minimal, although CR was attained by two patients in each group. In patients with melanoma, the response was seen only at the level of cutaneous metastases. In our series, no consistent response was observed in the small groups of patients with osteogenic sarcoma and carcinoma of uterine cervix. Chondrosarcoma and clear cell carcinoma of the kidney appeared to be resistant.

Besides the patients with the above-mentioned tumors, a miscellaneous group of patients with advanced neoplasms was treated during a phase II study (26,27). It is noteworthy that responses were obtained in patients with various malignant tumors of the peripheral nervous system (five of six patients), choriocarcinomas of uterus (two of two), and malignant thymomas (one of two).

Response in Relation to Dose Schedule

Table 8 reports the breakdown of respónse in relation to the six dose schedules employed. In order to clinically analyze a meaningful number of patients, cell types were grouped together whenever possible (eg, sarcomas, lymphomas, and bronchogenic, thyroid, and ovarian carcinomas) and objective improvement was also considered. Despite the small number of patients treated with some of the schedules, this type of analysis generally failed to show that there was a percent difference in the response rates among the different dose schedules. If one compares all responses obtained using the first four schedules with those achieved using schedules E and F, a percent difference in favor of the every-3-week treatment schedules becomes evident in breast cancer, melanoma, and thyroid carcinoma. The opposite finding was observed in testicular tumors and in neuroblastoma. The percent of CR is, however, different in the two groups: A + B + C + D = four of 179 patients (2%) versus E + F = 19 of 196 patients (10%). The incidence of CR was about the same between schedule E (nine of 90, 10%) and schedule F (ten of 106, 9%). In conclusion, despite the limitations of this type of analysis, the overall response rate of the present series does not appear to be strictly dependent on the dose schedule. The intermittent high-dose schedules, however, do seem to produce a higher percent of CRs.

Response to Combination Chemotherapy

Breast Cancer

In 1973, a controlled pilot study in advanced disease was undertaken to assess the effectiveness of the combination of adriamycin plus vincristine and to evaluate prednisone when given in addition to this combination (11). Adriamycin (40-75 mg/m^2) was administered on Day 1 of each course and was repeated at 3-week intervals. Vincristine was given on Days 1 and 8 at a dose level of 1.4 mg/m^2, and prednisone was given at a dose level of 100 mg/m^2 for the first 5 consecutive days. The combination of adriamycin plus vincristine produced CR or PR in more than one half of the patients, but prednisone was of no value in this particular treatment regimen (57% with prednisone versus 66% without). Soft tissue metastases were the most responsive site of involvement (75% response rate in each treatment arm) and partial recalcification of osteolytic lesions was observed in a total of 25% of the patients. The median duration of response was 4.5 months for each treatment group. Toxic manifestations were within acceptable limits and patients received 81% of the projected dose of adriamycin, 84% of vincristine, and 90% of prednisone.

TABLE 7.—Neoplastic diseases with poor or no response to adriamycin

Disease	No. of patients	CR + PR (%)	No. of patients with CR	Response rate (%)	Median duration of response (mos)
Gastrointestinal carcinoma	19	21	0	42	2.5
Melanoma	23	13	2	26	3
Head and neck carcinoma (epidermoid)	39	10	2	31	2
Osteogenic sarcoma	8	0		25	1
Cervical carcinoma	4	1/4		2/4	2
Chondrosarcoma	5	0		0	
Renal carcinoma	5	0		0	

TABLE 8.—Breakdown of response rate in relation to dose schedules

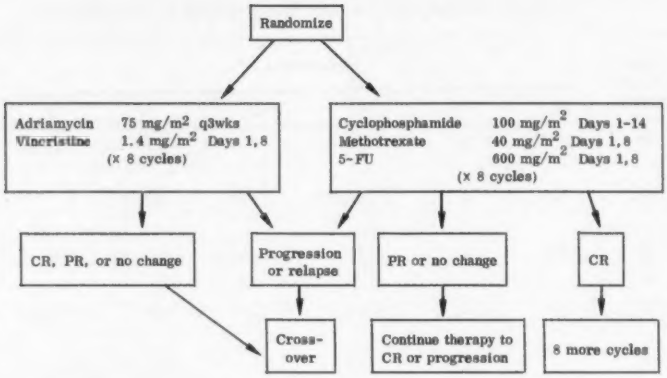
Tumor type	Total No. of patients	Dose schedules*						Total response*	
		A	B	C	D	E	F	A+B+C+D	E+F
Sarcomas (all types)	89	2/5	2/2	9/19	10/15	13/27 (1)	9/21 (1)	23/41 (56%)	22/48 (2) (46%)
Lymphomas (all types)	63	2/3 (1)	5/7	19/22 (2)	19/25	5/6 (2)	—	45/57 (3) (79%)	5/6 (2) (83%)
Lung (all types)	49	0/3	—	1/1	6/13 (1)	7/15 (1)	8/17	7/17 (1) (41%)	15/32 (1) (47%)
Head and neck (epidermoid)	39	1/2	—	1/4	1/4	2/3	7/26 (2)	3/10 (30%)	9/29 (2) (31%)
Breast	28	—	—	2/7	1/7	5/10 (1)	3/4 (1)	3/14 (21%)	8/14 (2) (57%)
Testicular (all types)	23	0/1	—	4/6	5/6	4/9 (1)	0/1	9/13 (69%)	4/10 (1) (40%)
Melanoma	23	0/1	0/2	—	0/2	1/4	5/14 (2)	0/5	6/18 (2) (33%)
Ovarian (all types)	18	—	—	—	—	4/8 (1)	4/10 (1)	—	8/18 (2) (44%)
Nasopharyngeal	16	—	—	3/4	1/4	2/3 (1)	2/5 (1)	4/8 (50%)	4/8 (2) (50%)
Neuroblastoma	16	1/1	1/1	3/4	3/4	2/5 (1)	0/1	8/10 (80%)	2/6 (1) (33%)
Thyroid (all types)	11	—	—	1/2	0/2	—	5/7 (2)	1/4 (25%)	5/7 (2) (71%)

*No. of patients responding/total No. treated; No. of patients with CR in parentheses.

TABLE 9.—Adriamycin plus vincristine versus cyclophosphamide, methotrexate, and 5-FU in the treatment of breast cancer: comparison of response

	Response rate (%) with—	
	Adriamycin and vincristine (52 patients)	Cyclophosphamide, methotrexate, and 5-FU (53 patients)
Type of response		
CR	8	11
PR	44	42
CR + PR	52	53
Overall	63	70
Site of response		
Breast	63	57
Skin	62	55
Nodes	74	70
Bone	27	21
Viscera	32	55
Median duration of response in mos (range)	7 (1-16)	6 (2-18)

Figure 1 presents the schema of the ongoing controlled trial designed in 1973 on the basis of the results achieved in the above-mentioned pilot study (12). This trial compares the combination of adriamycin and vincristine to the combination of cyclophosphamide, methotrexate, and 5-FU. Table 9 reports the results obtained at the time of the present writing. No statistical difference was observed be-



Stratification according to:

Menopausal status: (a) 0-1 yr, (b) 1-5 yrs, (c) 5-10 yrs, (d) > 10 yrs.
Free interval: (a) simultaneous, (b) 1-5 yrs, (c) > 5 yrs.
Dominant lesion: (a) soft tissue, (b) visceral, (c) osseous.

FIGURE 1.—Outline of controlled study in advanced breast cancer.

tween the two combinations in terms of type of response and median duration of response. After crossover, the secondary treatment produced a response rate of 31% with cyclophosphamide, methotrexate, and 5-FU, and 20% with adriamycin plus vincristine. Both treatments were fairly well tolerated, and the overall average percent of the optimal dose given was as follows: adriamycin, 84%; vincristine, 80%; cyclophosphamide, 80%; methotrexate, 82%; and 5-FU, 82%.

Another ongoing trial deals with combination chemotherapy (adriamycin plus vincristine) in patients with untreated inoperable breast cancer with local-regional (T_{3b}-T_4) extent. The outline of the study is shown in figure 2. The main reason for starting this combined approach was due to the poor control achieved in the past with both kilovoltage and megavoltage radiotherapy, with or without a subsequent radical mastectomy. A recent retrospective review conducted at our Institute with 454 patients from 1968 to 1972, showed that the overall actuarial survival rate was 42% at 3 years and 38% at 5 years. Furthermore, relapse occurred in 15% of the patients within 6 months after the start of irradiation treatments, and in 36% of the patients within 12 months (32). This new study was activated in the fall of 1973 with the hope of improving the relapse-free period by sequentially combining chemotherapy and radiotherapy. Chemotherapy was started before the irradiation treatments because new manifestations of disease occurred in areas distant from the irradiation fields in 68% of the patients (32). The preliminary results of the remission-induction phase reveal that the overall response rate after four cycles of adriamycin plus vincristine was 86% (CR 16%; CR + PR 72%) and that 74% of the patients were considered to be in clinical CR at the completion of the irradiation program (33). At the time of this report, it was too early to evaluate the role of maintenance chemotherapy. Considering the available data, the combined chemotherapy-radiotherapy approach seems to reduce the incidence of early relapses (18%) when retrospectively compared to the radiotherapy alone approach (36%). A larger number of patients and a longer period of observation are required to confirm these findings.

Other Solid Tumors

The comparative preliminary therapeutic results of combination chemotherapy in patients with advanced carcinomas of the uterine cervix and testicle as well as in those with soft tissue sarcomas are presented in tables 10-12. In patients with epidermoid carcinoma of the cervix, the combination of

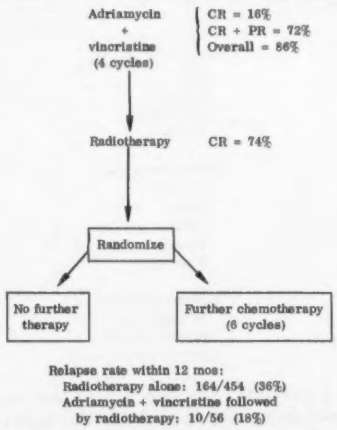


FIGURE 2.—Outline of study and preliminary results in inoperable (T_{3b}-T_4) breast cancer.

adriamycin plus bleomycin yielded a better response rate than the combination of cyclophosphamide plus vincristine (34). However, this latter combination produced a longer median duration in responsive patients. Regressions were observed in all types of metastases in patients treated with adriamycin plus bleomycin, while only patients with pulmonary and intrapelvic lesions responded to cyclophosphamide plus vincristine. In the group of patients receiving adriamycin plus bleomycin, the median survival time from the start of chemotherapy was longer in responders (8 months) as compared to nonresponders (3 months). This difference was not observed in the other treatment group.

In embryonal carcinoma of the testicle (table 11), both combinations appeared almost ineffective. In our opinion, the main reason for this was patient selection. In fact, practically all patients included in this trial also had extensive intra-abdominal node involvement. Therefore, the failure to achieve a significant regression at the level of retroperitoneal adenopathies prevented, in practically all instances, classification of patients in PR or CR groups despite significant and even complete regression of other lesions (eg, those of the lung).

In the group of patients with tissue sarcomas (table 12), the combination of adriamycin plus NSC-45388 proved to be definitely superior to the triple combination of cyclophosphamide, methotrexate, and vincristine (35). The incidence of CR plus PR was similar to that reported by Gottlieb et al (15). The percent of projected dose given was: 71% adriamycin; 70% NSC-45388; 59% cyclophosphamide; 61% vincrisine; and 60% methotrexate. After cross-

TABLE 10.—Controlled study in advanced carcinoma of the uterine cervix

Regimen	Drug	Dose and schedule*	No. of patients	CR + PR (%)	Overall response rate (%)	Median duration of response in mos (range)
1	Adriamycin	75 mg/m², iv, Day 1	15	20	53	2.5+ (1-12)
	Bleomycin	15 mg/m², iv, Days 1,8				
2	Cyclophosphamide	1.2 g/m², iv, Day 1	20	10	30	5.5+ (1-8)
	Vincristine	1.4 mg/m², iv, Days 1,8				

*Cycles repeated q3wks; crossover at time of progression.

TABLE 11.—Controlled study in advanced testicular carcinoma

Regimen	Drugs	Dose and schedule*	No. of patients	CR + PR (%)	Overall response rate (%)	Median duration of response in mos (range)
1	Adriamycin	50 mg/m², iv, Day 1				
	Methotrexate	20 mg/m², iv, Days 1,8	16	13	44	3
	Vincristine	1.4 mg/m², iv, Days 1,8				
2	Mithramycin	1.5 mg/m², iv, Days 1,4,8				
	Vinblastine	10 mg/m², iv, Days 1,8	13	0	15	1.5
	Bleomycin	20 mg/m², iv, Days 1,8				

*Cycles repeated q3wks; crossover at time of progression.

TABLE 12.—Controlled study in advanced soft tissue sarcoma

Regimen	Drugs	Dose and schedule*	No. of patients	CR + PR (%)	Overall response rate (%)	Median duration of response in mos (range)
1	Adriamycin	60 mg/m², iv, Day 1	18	50	72	4 (1+ -6)
	NSC-45388	250 mg/m², iv, Days 1-5				
2	Cyclophosphamide	1 g/m², iv, Day 1				
	Methotrexate	20 mg/m², iv, Days 1,8	19	11	58	3 (2-4)
	Vincristine	1.4 mg/m², iv, Days 1,8				

*Cycles repeated q3wks; crossover at time of progression.

over, response occurred in none of 13 patients receiving adriamycin plus NSC-45388 as compared to two of eight of those treated with cyclophosphamide, vincristine, and methotrexate.

Table 13 presents the results of a controlled trial in advanced ovarian carcinoma. CR plus PR as well as the overall response rate were higher after adriamycin treatment than after treatment with melphalan, although melphalan produced a longer duration of response. The number of cycles required to achieve the initial tumor regression was fewer in the group that received adriamycin (median, one cycle) than in the group receiving melphalan (median, two cycles). Regressions were observed in all types of lesions including retroperitoneal nodes, with the exception of lesions of the liver and external genitalia. The average total dose was 88% for melphalan and 87% for adriamycin. After crossover, melphalan induced response in four of 12 evaluable patients as compared to four of six who received adriamycin (36).

Childhood Neoplasms

The response achieved with adriamycin, cyclophosphamide, and vincristine in 11 of 19 (59%) consecutive previously untreated patients with stage IV neuroblastoma was reported in detail in 1974 (14). Table 14 outlines the response in relation to the sites of the lesions. The main reason why no patients were classified as complete responders was that a recalcification of bone metastases was not observed. The median duration of regression was 11+ months. The median survival time of responsive patients was about six times longer than that of unresponsive patients (fig 3). Figure 4 compares the survival time of the total group of 19 patients who received adriamycin, cyclophosphamide, and vincristine with that of 12 previously untreated patients with stage IV neuroblastoma who received adriamycin alone as primary therapy. The data show the combination chemotherapy to be superior, although, as previously reported (14), the difference in the median survival

TABLE 13.—Controlled study in stages III and IV ovarian carcinoma

Regimen	Drugs	Dose and schedule*	No. of patients	CR + PR (%)	Overall response rate (%)	Median duration of response in mos (range)
1	Adriamycin	75 mg/m², iv, Day 1, q3wks	19	42	58	3 (2-10)
2	Melphalan	6 mg/m², orally, Days 1-5, q4wks	19	26	42	6.5 (1-7.5)

*Crossover at time of progression.

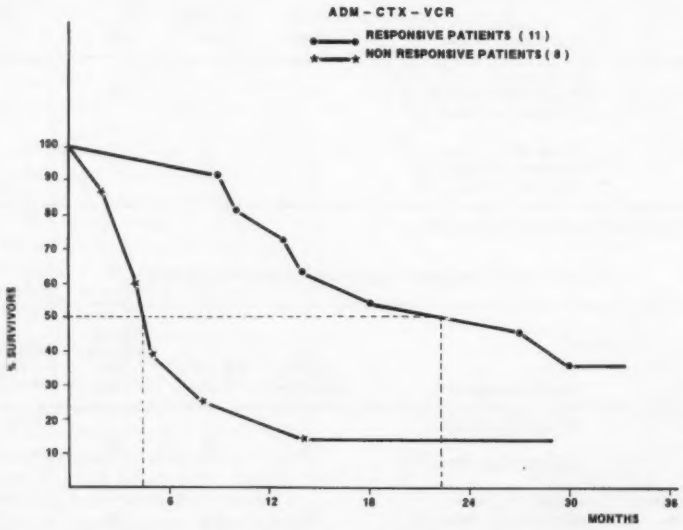


FIGURE 3.—Stage IV neuroblastoma treated with adriamycin (ADM), cyclophosphamide (CTX), and vincristine (VCR). Comparison of survival time of responders and nonresponders.

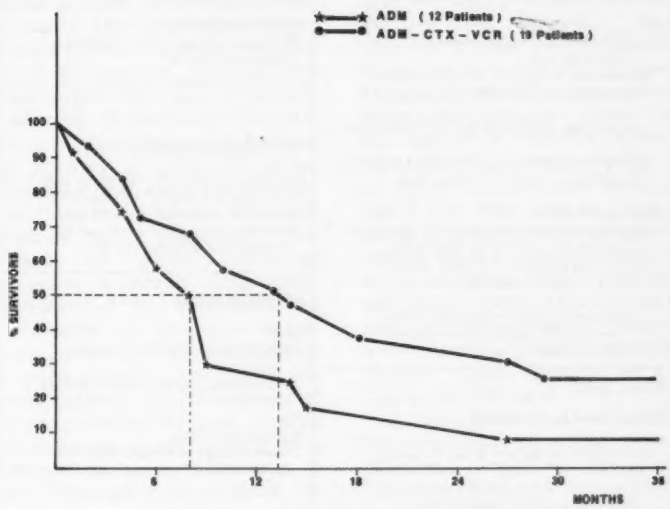


FIGURE 4.—Stage IV neuroblastoma. Comparison of survival time of patients receiving adriamycin (ADM), cyclophosphamide (CTX), and vincristine (VCR) and those primarily treated with ADM alone.

time of patients with neuroblastomas treated with combination chemotherapy was not statistically significant when retrospectively compared with that of patients who received single-agent therapy in sequence.

The recent therapeutic programs for solid tumors in children are outlined in figure 5. A combined therapeutic approach is planned for all forms of disease except stage IV neuroblastoma. In order to carry out a prolonged treatment program and to avoid the potential cardiac toxicity, sequential alternating cycles of combination chemotherapy have been designed for use in localized Ewing's sarcoma and rhabdomyosarcoma as well as in advanced Wilms' tumor. New quadruple combinations are designed for treatment of Ewing's sarcoma, rhabdomyosarcoma, and neuroblastoma. To the triad of cyclophosphamide, vincristine, and adriamycin, CCNU was added to treat both types of sarcomas, and NSC-45388 was added for treatment of neuroblastoma because of the preliminary evidence of the therapeutic activity of these new agents in childhood neoplasms (37). Although the initial results appear promising in all types of tumors, the number of patients entered into the study and the followup period are still too limited to provide meaningful information. However, all five children with osteogenic sarcoma are alive and free of disease after a median followup period of 6 months from the start of treatment, and both patients with diffuse Wilms' tumor have achieved CR.

TABLE 14.—Therapeutic response in stage IV neuroblastoma with adriamycin, cyclophosphamide, and vincristine

Site of lesions	Total No. of patients	No. of patients with—				Response rate (%)
		No response	Objective improvement	PR	CR	
Thorax	2	1	1			50
Abdomen	12	4	1	6	1	66
Bone	12	12*				0
Bone marrow	3	1			2	66
Lymph nodes	13	8	2	1	2	38
Skin	2			1	1	—
Lung	2	2				0
Liver	1	1				0

*No recalcification.

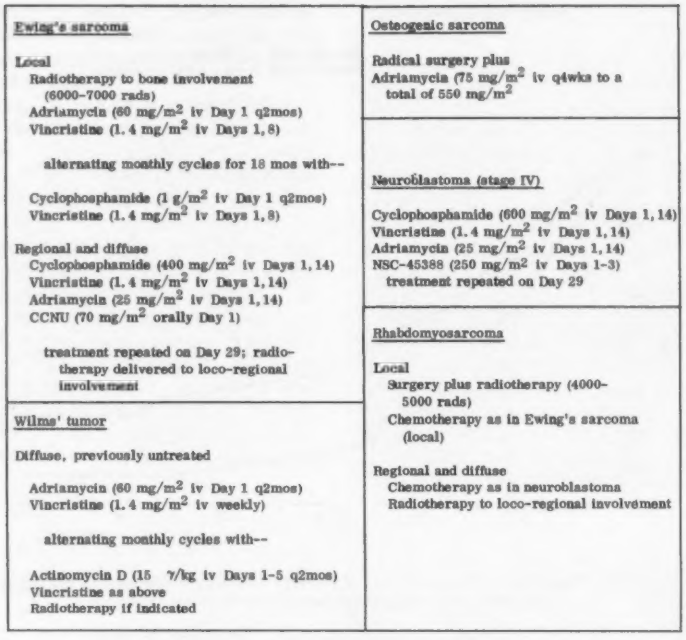

Ewing's sarcoma
Local
Radiotherapy to bone involvement (6000–7000 rads)
Adriamycin (60 mg/m² iv Day 1 q2mos)
Vincristine (1.4 mg/m² iv Days 1, 8)
alternating monthly cycles for 18 mos with--
Cyclophosphamide (1 g/m² iv Day 1 q2mos)
Vincristine (1.4 mg/m² iv Days 1, 8)
Regional and diffuse
Cyclophosphamide (400 mg/m² iv Days 1, 14)
Vincristine (1.4 mg/m² iv Days 1, 14)
Adriamycin (25 mg/m² iv Days 1, 14)
CCNU (70 mg/m² orally Day 1)
treatment repeated on Day 29; radiotherapy delivered to loco-regional involvement
Wilms' tumor
Diffuse, previously untreated
Adriamycin (60 mg/m² iv Day 1 q2mos)
Vincristine (1.4 mg/m² iv weekly)
alternating monthly cycles with--
Actinomycin D (15 γ/kg iv Days 1–5 q2mos)
Vincristine as above
Radiotherapy if indicated
Osteogenic sarcoma
Radical surgery plus
Adriamycin (75 mg/m² iv q4wks to a total of 550 mg/m²
Neuroblastoma (stage IV)
Cyclophosphamide (600 mg/m² iv Days 1, 14)
Vincristine (1.4 mg/m² iv Days 1, 14)
Adriamycin (25 mg/m² iv Days 1, 14)
NSC-45388 (250 mg/m² iv Days 1–3)
treatment repeated on Day 29
Rhabdomyosarcoma
Local
Surgery plus radiotherapy (4000–5000 rads)
Chemotherapy as in Ewing's sarcoma (local)
Regional and diffuse
Chemotherapy as in neuroblastoma
Radiotherapy to loco-regional involvement

FIGURE 5.—New ongoing studies in childhood solid tumors.

Figure 6 presents the induction phase of therapy for patients with acute lymphoblastic leukemia and nonHodgkin's lymphoma. In patients with leukemia, five of eight achieved CR for a median duration of 13+ months. In patients with lymphomas, six of six achieved CR but relapse occurred in two children after 1 and 3 months, respectively, from the time of remission.

DISCUSSION

The data available from our studies confirm once more that adriamycin is an effective new drug that is able to produce objective response in a wide range of neoplastic diseases. The activity of adriamycin, in fact, encompasses the major types of cancer in adults and children, with the exception of renal cancer, melanoma, and gastrointestinal carcinomas. In the latter two neoplasms, however, the data of new studies may possibly change the above statement. In fact, our most recent findings indicate that malignant melanoma is not entirely unresponsive to adriamycin, and unpublished results of Moertel (USA) and Brugarolas (Spain) show that interesting regressions are being observed in gastric cancer.

From the therapeutic point of view, the most important finding from studies with adriamycin is that this drug has probably contributed more than any other available agent to overcoming the barrier of solid tumors. In fact, many types of cancers known in the past to be poorly or not responsive to single agents and to combination chemotherapy (eg, soft tissue sarcomas, osteogenic sarcoma, carcinomas of urinary bladder and thyroid, mesothelioma) showed a remarkable response rate when treated with adriamycin alone and in combination. Furthermore, in other forms of advanced neoplasms (eg, histiocytic lymphoma, breast cancer, neuroblastoma, Ewing's sarcoma, Wilms' tumor) there is initial evidence of more successful control when adriamycin is added to conventional treatments. In other diseases known to be either very responsive to chemotherapy (eg, Hodgkin's disease, lymphocytic lymphoma, acute leukemia, ovarian carcinoma) or moderately responsive (eg, carcinomas of testicle, lung, head and neck, uterine cervix), it remains to be demonstrated with certainty whether adriamycin can really improve the results being obtained with standard regimens.

In our efforts to show that adriamycin is a useful agent in the treatment of many forms of tumors, a number of problems remain open to future studies.

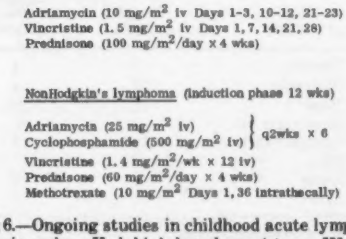

Acute leukemia (induction phase 4–6 wks)
Adriamycin (10 mg/m² iv Days 1–3, 10–12, 21–23)
Vincristine (1.5 mg/m² iv Days 1, 7, 14, 21, 28)
Prednisone (100 mg/m²/day x 4 wks)
NonHodgkin's lymphoma (induction phase 12 wks)
Adriamycin (25 mg/m² iv)
Cyclophosphamide (500 mg/m² iv)
q2wks x 6
Vincristine (1.4 mg/m²/wk x 12 iv)
Prednisone (60 mg/m²/day x 4 wks)
Methotrexate (10 mg/m² Days 1, 36 intrathecally)

FIGURE 6.—Ongoing studies in childhood acute lymphoblastic leukemia and nonHodgkin's lymphoma (stages III and IV).

The first problem concerns the optimal dose schedule. The pharmacokinetic studies (38-40) have confirmed, on a scientific basis, that the intermittent high-dose schedules have a rational foundation. The long plasma half-life of adriamycin provides the explanation for the decreased incidence of severe hematosuppression and oral ulcerations with the every-3-week schedule as compared to schedules with shorter intervals between courses. The same studies have also emphasized that since the hepatobiliary excretion of adriamycin is about 40%, the dose level must be reduced in the presence of impaired liver function to avoid potential life-threatening toxicity due to delayed drug elimination (38,40). With substantial dose reduction, the therapeutic efficacy is similar to that seen in patients with normal liver function. The relationship between dose level or dose schedule and tumor response is not yet entirely defined. The importance of dose-response evaluation is crucial because cardiac toxicity limits the long-term use of adriamycin. In a controlled study with four dose levels, O'Bryan et al (41) concluded that the 75-mg/m^2 iv bolus given every 3 weeks was statistically superior to doses of 50, 45, and 25 mg/m^2. In our experience, however, the breakdown of tumor response in relation to six dose schedules failed to show the superiority of high-dose schedules except in a few neoplasms. However, the analysis indicated that the high-dose schedules were superior in inducing CR. In our series, no difference in tumor response was observed between the two every-3-week schedules, ie, single iv bolus or total dose given over a 3-day period. Since cardiomyopathy may also be related to the level of single doses, it should be recalled that this toxic manifestation was more often encountered in our series in patients who received the high intermittent dose schedules. It is important to realize, however, that in our series the incidence of cardiomyopathy was 2.3% on schedule F where the highest single doses were used and 4.3% on schedule E where 3-day courses at lower single doses of drug per day were utilized. These data would suggest that the level of each single dose was less significant than the total dose of drug per course or per unit time. Probably all problems related to dose-response evaluation will be overcome by the fact that adriamycin is being introduced into more and more different types of combinations. As shown in our various trials, the intermittent administration of adriamycin with standard agents not only can be safely tolerated, but can produce good PRs and CRs that are comparable, if not superior, to those produced by conventional multiple-drug treatments.

The second important problem is how to avoid or limit the incidence of cardiomyopathy. Practically all investigators agree that the incidence of cardiac failure markedly increases after cumulative doses of 550-600 mg/m^2. The clinical picture is usually characterized by either an abrupt or more often insidious onset, cardiomegaly, biventricular failure associated or preceded by an increased ratio of pre-ejection period to left ventricular ejection time (PEP/LVET), and low QRS voltage (42). Since there are no convincing data supporting the hypothesis that the concomitant administration of strophanthin or digitalis can decrease the incidence of cardiomyopathy, the only preventive measure consists in stopping the drug administration before the cumulative dose of 550 mg/m^2 is reached. Clinicians should also be aware that a history of prior cardiovascular disease or prior radiotherapy to areas encompassing the heart are risk-factors, and cardiomyopathy may occur in these instances after total doses below 450 mg/m^2 (43,44). Again, most if not all problems related to cardiomyopathy may be solved by combining adriamycin with other agents. As shown in our studies, an appropriately designed combination can avoid cardiac toxicity, provided that during the induction phase the total dose administered remains below the risk level.

A third problem involves the present indications for administering adriamycin alone to patients with advanced neoplasms. The contemporary era of combination chemotherapy is rapidly changing the conventional therapeutic approach for treatment of almost all types of neoplastic disease. Therefore, since adriamycin is effective in a wide range of tumors, it appears logical to include this agent in many new combined drug regimens in an effort to improve the therapeutic results. Our studies, and those of other investigators (15-19), provide some good examples of new developing strategies. With the present knowledge of therapy for advanced neoplastic diseases, however, adriamycin alone is still indicated as primary therapy in thyroid carcinomas and in mesotheliomas where no other effective single agents or combinations are available. The administration of adriamycin as a single agent is also indicated in patients with neoplasms susceptible to relapse after conventional chemotherapy. In these cases, after having reached the cumulative dose of 600 mg/m^2, treatment can only be continued if the response warrants the risk and if there are no other effective drugs available for trial (5).

A fourth problem concerns the best type of combination with adriamycin for each type of responsive tumor. Presently, there is no definitive answer to this question. A large body of data is showing that adriamycin is synergistic in laboratory animals with some standard agents such as NSC-45388, vincristine, methotrexate, and cyclophosphamide. These findings provide a rational foundation for planning several types of effective combination regimens. A few examples in malignant lymphomas (13,-18,19,31), sarcomas (15), and breast cancer (11,-12,16,17) are already available in the literature. Only time will indicate which will be the treatment of choice to be recommended for each tumor type in general clinical practice. We would like to emphasize our current approach which consists of the development, through controlled trials, of noncross-resistant combinations. The sequential administration of effective combinations may result in an im-

proved control of susceptible neoplastic diseases provided the proper selection of patients and the timing between courses are also considered.

The final problem is related to the present role of adriamycin in adjuvant treatments. A conservative approach would tend to exclude, for the moment, a general indication for adriamycin as adjuvant therapy until we know more about cardiomyopathy and carcinogenicity. There are already published reports, however, showing that adjuvant treatment with adriamycin alone and in combination has dramatically improved the relapse rate in patients with osteogenic sarcoma (20,22,23), with results comparable to those obtained with methotrexate (45). Similar encouraging data are being obtained in other childhood neoplasms (21). We suggest that adriamycin not only can be employed as adjuvant therapy for tumors in which there is a high risk of early dissemination, but that adriamycin is also capable of an almost unique therapeutic role in diseases such as sarcomas of the bone and soft tissue, histiocytic lymphoma, and carcinomas of the thyroid and urinary bladder. In other types of neoplasms, such as breast cancer, it probably would be wiser to wait for the long-term results of controlled studies designed with standard drugs (46,47). In any case, the dose schedule of adriamycin should be cautiously planned to avoid cumulative toxicity which can occur after prolonged administration.

In conclusion, available data place adriamycin in the center of contemporary medical therapy for a number of neoplastic diseases and allow us to hope that in the near future a better knowledge of the use of this drug will contribute to new useful therapeutic approaches.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
Bleomycin: NSC-125066
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU
Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride; L-PAM
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
NSC-45388: CAS reg. No. 4342-03-4; 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide; DTIC
Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-
Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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An Overview of Studies With Adriamycin (NSC-123127) in the United States[1]

Ronald H. Blum[2,3,4]

The Division of Cancer Treatment (DCT), National Cancer Institute (NCI), through the Farmitalia Company, has supplied adriamycin for clinical trials in the United States since 1970. During the past 4 years, cooperative chemotherapy study groups, cancer centers, and independent investigators have been studying adriamycin in patients with advanced metastatic cancer. In this report, compiled data will be presented from published as well as unpublished data on file with Cancer Therapy Evaluation, DCT, NCI. This information has been collected as part of the Investigational Drug Application and is on file in compliance with Food and Drug Administration regulations. Only investigations from the United States are reported in this paper.

The data base that follows must be considered in the context of the inherent limitations of this type of review. The studies reported are uncontrolled. The parameter of response rate is an approximation subject to the biologic variability of tumors, the heterogeneity of patient populations, and the marked differences in approach of clinical investigators. Overall response rates represent pooling of not necessarily comparable data.

Generalizations about the patient population must also be considered (fig 1). Most patients had advanced metastatic tumors. Evaluable patients reported are from phase II activity-seeking studies. By definition, only patients with measurable tumors are included. Most patients had failed conventional chemotherapy.The evaluable patients reported had an adequate trial, usually completing more than one or two courses of the drug.

Response is defined as a greater than 50% regression of a measurable lesion for at least 1 month. The unweighted response rate is calculated from the number of patients responding divided by the number of evaluable patients.

Several dose schedules have been utilized (table 1). Adriamycin is usually given as a rapid infusion through a running intravenous (iv) line. The most commonly used dose schedule is 60-90 mg/m^2 repeated every 21 days. An alternative intermittent schedule is 20-30 mg/m^2/day for 3 days repeated every 21 days. Although there are no hard therapeutic or toxicologic data to support this intermittent dose schedule, the rationale for its use is twofold: (a) Adriamycin has a prolonged serum half-life of approximately 26-48 hours with a detectable amount of drug persisting in the serum for more than 1 week. (*b*) By reducing the frequency of administration, the probability of accidental extravasation is also reduced (1). Alternative drug schedules include 0.4 mg/kg/day on Days 1, 2, and 3 and repeated on Days 8, 9, and 10, with recycling after 14 days. Adriamycin has also been given weekly at a dose of 1 mg/kg/day.

Patient characteristics
1. Advanced metastatic tumor
2. Measurable tumor
3. Conventional treatment failures
4. Adequate trial

Definition of response
1. Objective tumor regression
   > 50% measurable decrease
   > 1-month duration
2. Unweighted response rate
   $\frac{\text{No. of patients responding}}{\text{No. of evaluable patients}} \times 100$

FIGURE 1.—Generalizations about patient population included in this review.

Haskell and co-workers have reported the use of intra-arterial adriamycin as part of a combined modality approach to extremity sarcomas (2). Their dose recommendation is 75 mg/m^2 intra-arterially infused over 72 hours.

Benjamin et al (1) have demonstrated prolonged adriamycin serum levels and increased toxicity in patients with impaired hepatocellular function (table 2). If their dose modification scheme is followed, adriamycin can be safely administered. Specifically, their regimen involves half doses in patients with mild hepatocellular dysfunction and quarter doses in patients with severe hepatocellular dysfunction.

Adriamycin is one of the most effective single agents against metastatic breast cancer (table 3). Of 390 patients reported, 37% responded. Virtually all patients had failed hormonal manipulation and conventional chemotherapy. The median duration of response in a group of 15 responders from a phase II study was 7 months, with a range of 1-11 months. The median interval to response was 2 months with a range of ½-7 months (4). Based on these observations, adriamycin is being utilized singly and in combination in early disease.

TABLE 1.—Adriamycin dose schedules

Route	Schedule
Intravenous	60-90 mg/m^2, q21d 20-30 mg/m^2 daily × 3, q21d 0.4 mg/kg Days 1,2,3,8,9,10, q2wks 1 mg/kg/day, q7d
Intra-arterial	75 mg/m^2 infused over 72 hrs

[1]Adriamycin: CAS reg. No. 23214-92-8.

[2]Sidney Farber Cancer Center, Harvard Medical School, Boston, Mass.

[3]I wish to thank E. Frei III for his review of the manuscript and Diane M. Simmons for preparation of the manuscript.

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TABLE 2.—Adriamycin dose reduction in patients with impaired liver function

Dose modification (% of intended dose)	Degree of impaired liver function	Bilirubin level (mg/100 ml)	Sulfobromophthalein (% retention)
100	Normal	<1.2	<5
50	Mild	1.2-3.0	5-15
25	Severe	>3.0	>15

TABLE 3.—Effects of adriamycin in breast cancer

Study group	Reference No.	No. of patients	No. of responses	Response rate (%)
Southwest Oncology Group	3-5	104	41	39
Albany Medical College and Roswell Park Memorial Institute	6	23	8	35
Mayo Clinic	7	20	9	45
Cancer Therapy Evaluation, DCT, NCI		243	86	35
Totals		390	144	37

TABLE 4.—Effects of adriamycin in sarcomas

Study group	Reference No.	No. of patients	No. of responses	Response rate (%)
Southwest Oncology Group	3	64	21	33
Sloan-Kettering Cancer Center	8	39	8	21
Baltimore Cancer Research Center, DCT, NCI	1	25	10	40
Mayo Clinic	9	20	2	10
Cancer Therapy Evaluation, DCT, NCI		151	40	26
Totals		299	81	27

The impressive therapeutic effect of adriamycin in sarcomas has dramatically altered the clinical approach to primary and metastatic disease (table 4). Of 299 patients reported, 27% responded. Comparison of the efficacy of adriamycin with that of other drugs is hampered by the relative paucity of data for the standard antitumor agents. This lack of data reflects the general resistance of this class of tumors.

For bone sarcomas, the 26% response rate for osteogenic sarcoma and the 45% response rate for Ewing's sarcoma demonstrate adriamycin's usefulness in these aggressive tumors (table 5). Chondrosarcomas are more resistant.

For soft tissue sarcomas, adriamycin induces tumor regression in about one third of the patients treated irrespective of cell type. An adriamycin combination using NSC-45388, vincristine, and cy-

TABLE 5.—Effects of adriamycin in sarcomas by cell type

Cell type	No. of patients	No. of responses	Response rate (%)
Bone sarcomas			
Osteogenic	78	20	26
Ewing's	51	23	45
Chondrosarcoma	8	1	—
Soft tissue			
Rhabdomyosarcoma	54	19	35
Leiomyosarcoma	28	10	36
Fibrosarcoma	20	6	30
Synovial cell sarcoma	15	5	33
Liposarcoma	13	4	31
Totals	130	44	34

clophosphamide[5] has increased the response rate to 60% as well as the duration of response (10).

Unresectable bronchogenic carcinoma, a very common but resistant tumor, demonstrates some sensitivity to adriamycin (table 6). Thirty-eight of 212 patients (18%) had a greater than 50% regression. Cell type data are limited. Small cell carcinoma and adenocarcinoma (33% and 21% response rate respectively) are somewhat more sensitive than epidermoid and large cell undifferentiated bronchogenic carcinomas (table 7).

Adriamycin is useful in patients with hematologic malignancies (table 8). Used as a single agent, adriamycin has resulted in a 32% response rate for Hodgkin's disease and a 40% response rate for non-Hodgkin's lymphoma.

Daunorubicin,[6] the original clinical analog of adriamycin, had definite activity in leukemia; adriamycin is at least as active as daunorubicin. In acute lymphocytic leukemia, 22% of the evaluable patients treated with adriamycin had complete responses. In acute nonlymphocytic leukemia, 11% of the evaluable patients had complete responses. These response rates were achieved in end-stage patients who had failed to respond to more standard drugs.

Twenty-five percent of multiple myeloma patients have demonstrated sensitivity to adriamycin. Renal impairment, often occurring in this disease, does not seem to exaggerate the pharmacologic effect of adriamycin.

In phase II activity-seeking studies, adriamycin demonstrates antitumor effect in a variety of adult and childhood solid tumors. Seventy-one patients with neuroblastoma have been treated with adriamycin and 21 (30%) had an objective response (table 9). Neuroblastoma is predominantly a pediatric tumor, but analysis by age in the patient population, a known clinical variable, is not available. Wilms' tumor is also sensitive to adriamycin. Sixty-five percent of 40 evaluable patients have demonstrated objective regression, with 15% complete responses.

In genitourinary tumors, the response rates are 22% for bladder cancer, 26% for testicular cancer, 16% for ovarian cancer, and 14% for prostatic cancer (table 10). It should be noted that the 16% response rate for ovarian cancer is lower than we previously reported. One third of the patients with epidermoid carcinoma of the cervix responded, but only 21% of the patients with epidermoid carcinoma of the head and neck responded (table 11). Gastrointestinal cancer, malignant melanoma, and renal cell carcinoma are resistant to adriamycin. The denominations are significant.

The toxic manifestations of adriamycin are dose related, predictable, and usually reversible (fig 2). Total alopecia is seen in most patients. Leukopenia is dose limiting with nadirs typically occurring 10-14 days after a single dose, but lasting only a few days with prompt recovery. Drug-induced anemia and thrombocytopenia are much less severe.

Anorexia, nausea, vomiting, and inanition are transient, lasting 12-48 hours after a single dose. There is a high degree of individual patient variation. Antiemetics have an ameliorating effect. Stomatitis can be severe, but is generally not dose limiting with the intermittent dose schedule.

Subcutaneous extravasation of adriamycin leads to profound tissue necrosis with sloughing of the skin. Extreme care must be taken to avoid this complication. The red anthracycline pigment is noted in the patient's urine immediately after administration. Hyperpigmentation of the skin, nails, and buccal mucosa has been seen in some patients, especially in blacks (12). Adriamycin can induce radiation recall with focal skin reactions (13). Also, some patients report increased lacrimation, occurring 5-15 days after a single dose.

[5]NSC-45388: CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-; DTIC.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

[6]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

TABLE 6.—Effects of adriamycin in lung cancer

Study group	Reference No.	No. of patients	No. of responses	Response rate (%)
Southwest Oncology Group	3	56	8	14
Roswell Park Memorial Institute	11	31	9	29
Baltimore Cancer Research Center, DCT, NCI	1	10	5	50
Sloan-Kettering Cancer Center	8	8	0	—
Cancer Therapy Evaluation, DCT, NCI		107	16	15
Totals		212	38	18

TABLE 7.—Effects of adriamycin in lung cancer by cell type

Cell type	No. of patients	No. of responses	Response rate (%)
Small cell carcinoma	18	6	33
Adenocarcinoma	38	8	21
Epidermoid carcinoma	22	4	18
Large cell undifferentiated carcinoma	26	1	4

TABLE 8.—Effects of adriamycin in hematologic cancer

Disease	No. of patients	No. of responses	Response rate (%)	Complete response rate (%)
Lymphoma				
Hodgkin's	75	24	32	
Non-Hodgkin's	165	66	40	
Leukemia				
Acute lymphocytic	235	86	37	22
Acute nonlymphocytic	82	25	30	11
Myeloma	20	5	25	

TABLE 9.—Effects of adriamycin in childhood solid tumors

Disease	No. of patients	No. of responses	Response rate (%)	Complete response rate (%)
Neuroblastoma	71	21	30	
Wilms' tumor	40	26 (6 complete)	65	15

TABLE 10.—Effects of adriamycin in genitourinary cancer

Carcinoma	No. of patients	No. of responses	Response rate (%)
Bladder	120	26	22
Testicular	42	11	26
Ovarian	88	14	16
Prostatic	51	7	14

TABLE 11.—Effects of adriamycin in solid tumors

Disease	No. of patients	No. of responses	Response rate (%)
Sensitive, epidermoid			
Head and neck	76	16	21
Cervix	27	9	33
Thyroid	22	9	41
Resistant			
Gastrointestinal	151	14	9
Melanoma	21	0	0
Renal cell	56	4	7

1. Alopecia
2. Myelosuppression: wbc > hematocrit > platelets
3. Gastrointestinal (acute)
4. Stomatitis
5. Cardiac
6. Extravasation: necrosis and slough
7. Other: red urine, skin pigmentation, lacrimation

FIGURE 2.—General toxic effects of adriamycin therapy.

Electrical
Immediate, transient
No dose relationship
Supraventricular tachyarrhythmias
ECG, ST-T wave changes

Pump
Delayed effect
Dose relationship (↑ > 550 mg/m^2)
Cardiomyopathy

FIGURE 3.—Cardiotoxicity of adriamycin therapy.

Conceptually, adriamycin cardiac toxicity should be divided into electrical effects and pump failure (fig 3). Electrical toxicity is reflected in electrocardiographic changes of supraventricular tachyarrhythmias and occasional ventricular extrasystoles and ST-T wave changes. These effects occur during the first few days after drug administration and rarely cause significant morbidity. There does not seem to be a dose relationship. Arrhythmia-induced mortality has not been reported. The pump failure is a delayed effect of cumulative doses, usually in excess of 550 mg/m^2.

In conclusion, as shown by this overview of trials in the United States, adriamycin has definite antitumor activity in adult and childhood solid tumors and hematologic malignancies. Adriamycin probably lacks clinical cross resistance to standard antineoplastic agents, and may be more effective in patients with less advanced disease. The ultimate role of adriamycin therapy in the control of cancer will depend on the results of properly designed prospective clinical studies using adriamycin as a single agent, in combination chemotherapy, and in combined modality approaches.

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Adriamycin (NSC-123127) Studies in Adult Patients[1]

Irwin H. Krakoff[2,3]

The early results in the treatment of cancer in adults with adriamycin at Memorial Hospital have been reported elsewhere (1,2). Since there have been differences between adults and children in both dosage schedules and responses, this paper will be concerned only with adults. The studies reviewed represent the efforts of members of the Solid Tumor, Clinical Chemotherapy, Hematology, and Lymphoma Services. The pediatric experience will be reviewed elsewhere in this symposium by Dr. Charlotte Tan et al (3).

The initial dosage exploration at Memorial Hospital was not a classic phase I study since substantial information was already available from Bonadonna et al (4,5) concerning tolerated dosage. In our initial studies we examined the effects of adriamycin when given by consecutive daily doses, usually for 3 days each week. However, as has been the case with other investigators, we concluded that single intermittent doses given once every 3 or 4 weeks appeared to be better tolerated and no less effective than daily doses. Thus, our later experiences concentrated on a dose schedule of 60-75 mg/m^2 given every 3 weeks.

As has previously been reported, the toxic effects of adriamycin are similar to those of other cytotoxic agents. Leukopenia, thrombopenia, and stomatitis have occurred regularly in patients considered to have received adequate therapy. Alopecia which occurred sporadically at low doses has been a regular phenomenon at doses presently considered to be in the therapeutic range and can be anticipated in virtually all patients. The most disturbing toxic manifestation and that which is most limiting in the use of adriamycin is cardiac toxicity, which has been encountered by us as well as by other investigators. Since that phenomenon will be the subject of a separate section of this symposium it will not be reviewed here.

Our initial trial of adriamycin was aimed at a wide spectrum of tumor types. These are summarized in tables 1-3. Although the response rates indicated in those tables were generally disappointing, there were indications of activity which have led to broader considerations of adriamycin in combination chemotherapy. Table 1 demonstrates impressive response rates only in carcinoma of the breast. In that disease, we noted a response rate of approximately 50% (category I, Karnofsky et al [6]). In a large group of bone and soft tissue sarcomas a response rate of nearly 20% was seen (table 2).

In acute leukemias, evaluation of single new agents is difficult because current success with cyclic combination chemotherapy precludes the trial of single new agents in early disease. Nevertheless, in patients with far-advanced, otherwise nonresponsive acute lymphoblastic leukemia, sufficient responses were seen (table 3) to warrant the consideration of adriamycin for inclusion in multiple-drug protocols. In the lymphomas an encouraging number of useful responses were seen even in far-advanced, heavily pre-treated disease. Thus, although our initial experience in an early phase II study did not reveal spectacular activity, sufficient responses were seen to warrant the inclusion of adriamycin in a number of multiple-drug programs where it appears to have contributed substantially to response rates both in our own experience and in the experience of other investigators.

Tables 4-9 summarize our experience with several multiple-drug regimens incorporating adriamycin. Prior experience with other regimens has clearly indicated the general superiority of multiple-drug chemotherapy over monochemotherapy. Table 4 summarizes the experience to date with a combination of cyclophosphamide, adriamycin, and 5-fluorouracil given cyclically in carcinoma of the breast. When this combination was used in patients who had had no prior cytotoxic chemotherapy, the response rate was greater than 60%. It is important to note, however, that in a small group of patients who had been treated with other cytotoxic agents before receiving this combination, the response rate was only 23%, confirming the principle that patients treated early in the course of the disease and before there has been extensive treatment with other agents are likely to do better.

Tables 5 and 6 summarize our experience with a combination of methyl-CCNU, adriamycin, and vincristine in a group of patients with bone and soft tissue sarcomas and another large group of patients with a variety of nonsarcomatous solid tumors. In both groups the response rate was 20%-25%, not substantially different from the results obtained with adriamycin alone, seeming to confirm the principle that a compound which is not active alone is unlikely to add to the efficacy of a combination in which it is included. In contrast, table 7 demonstrates a regimen including vincristine, high-dose methotrexate with citrovorum factor rescue, adriamycin, and actinomycin D in sarcomas. Although the number reported here is small, the response rate appears to be significantly better than with the combination summarized in tables 5 and 6 or with adriamycin alone. This probably reflects the additive effects of methotrexate and actinomycin D, both

[1]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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of which are known to have some activity in this group of tumors.

It is now generally agreed that intensive multiple-drug cyclic chemotherapy, initiated promptly, provides the best opportunity for complete and prolonged remission in acute lymphoblastic leukemia. Table 8 summarizes the experience of the Memorial Sloan-Kettering Cancer Center with the complex protocol L10, which includes adriamycin. As the table indicates, the remission rate was 85% at the time of this report. It is impossible to assess the contribution of any single element of this regimen to the remission rate. However, the evidence that adriamycin is active alone in even very far-advanced disease suggests that it may be contributing substantially in this protocol.

Table 9 summarizes the results of a complex multiple-drug regimen including adriamycin which was studied in the treatment of non-Hodgkin's lymphoma. Although it was possible to achieve 70% remissions in histiocytic lymphomas, these tended to be relatively brief and the bone marrow toxic effects were substantial. Therefore, this particular protocol has been terminated although it appears to indicate the potential for combinations of this type in non-Hodgkin's lymphomas.

As a result of convincing evidence that adriamycin in multiple-drug combinations has been effective in a variety of tumor types, we are presently engaged in evaluating it in tumor types not studied before, as well as attempting to improve upon protocols already shown to be effective.

Clearly, it appears that adriamycin represents an important useful addition to the roster of drugs available to treat certain specific neoplasms. At present it appears to be most useful as one agent in combination regimens. It seems likely that its full range of activity and the optimum methods for using it have not yet been completely defined.

TABLE 1.—Adriamycin studies in adults with solid tumors

Disease	Total No. of patients	No. of evaluable patients	No. of responses* (duration in mos)
Head and neck carcinoma	14	12	0
Mesothelioma, lung	1	0	0
Lung carcinoma	23	17	0
Bladder carcinoma	27	19	1(7)
Cervical carcinoma	16	12	2 (2, 3)
Breast carcinoma	30	25	13 (2-9)
Melanoma	3	2	0
Neuroblastoma	3	2	0
Miscellaneous tumors	81	59	0

*Category I (A, B, C) responses evaluated according to Karnofsky et al (6).

TABLE 2.—Adriamycin studies in adults with sarcomas

Disease	Total No. of patients	No. of evaluable patients	No. of responses* (duration in mos)
Ewing's	8	6	2 (7,8)
Osteogenic	8	5	1 (1½)
Alveolar	2	2	0
Lymphangiosarcoma	3	1	0
Fibrosarcoma	5	3	0
Fibrous xanthoma	3	2	0
Leiomyosarcoma	20	14	2 (3,8+)
Liposarcoma	8	5	1 (1½)
Myosarcoma	1	1	0
Neurilemoma	3	3	0
Rhabdomyosarcoma	23	12	2 (1, 4)
Synovial	16	11	4 (3-4)
Miscellaneous	3	2	0
Total	103	67	12

*Category I (A, B, C) responses evaluated according to Karnofsky et al (6).

TABLE 3.—Adriamycin in adults with leukemias and lymphomas

Disease	Total No. of patients	No. of evaluable patients	No. of responses* (duration in mos)
Acute leukemia			
Lymphoblastic	11	11	4 GPR's† (¾-8)
Myelomonoblastic	5	4	0
Leukosarcoma	5	4	0
Hodgkin's disease	21	14	0
Lymphosarcoma	12	6	2 (1,2)
Lymphosarcoma, predominantly solid	5	3	3 (3-20)
Reticulum cell sarcoma	24	14	6 (1-2½)
Kaposi's sarcoma	4	2	2 (7, 9+)
Lymphoma, cutis	2	1	1 (17)

*Category I (A, B, C) responses evaluated according to Karnofsky et al (6).
†GPR = good partial remissions.

TABLE 4.—Adriamycin in combination with cyclophosphamide and 5-fluorouracil in adults with breast carcinoma

	Total No. of patients	No. of evaluable patients	No. of responses* (%)
No prior chemotherapy	40	31	19 (61)
Prior chemotherapy	18	13	3 (23)

*Category I (A, B, C) responses evaluated according to Karnofsky et al (6).

TABLE 5.—Adriamycin in combination with methyl-CCNU and vincristine in adults with sarcomas

Disease	Total No. of patients	No. of evaluable patients	No. of responses* (duration in mos)
Synovial	3	1	1 (6)
Alveolar soft part	2	2	0
Chondrosarcoma	1	—	—
Fibrosarcoma	6	3	—
Leiomyosarcoma	20	15	3 (3+-10)
Liposarcoma	4	3	2 (3, 12)
Mesothelioma	4	1	0
Rhabdomyosarcoma	6	6	2 (7, 7+)
Spindle cell	10	8	1 (5+)
Ewing's	1	1	0
Osteogenic	7	6	1 (4½)
Total	64	46	10

*Category I (A, B, C) responses evaluated according to Karnofsky et al (6).

TABLE 6.—Adriamycin in combination with methyl-CCNU and vincristine in adults with solid tumors

Disease	Total No. of patients	No. of evaluable patients	No. of responses* (duration in mos)
Head and neck carcinoma	17	9	5 (4+-7)
Lung carcinoma	38	22	4 (2-10+)
Breast carcinoma	6	5	1 (4+)
Stomach carcinoma	3	1	0
Liver carcinoma	4	3	0
Large bowel carcinoma	22	11	0
Kidney carcinoma	37	23	5 (2-17+)
Bladder carcinoma	8	5	2 (5, 10)
Uterine carcinoma	1	1	
Testicular carcinoma	26	15	1 (6)
Embryonal and/or teratocarcinoma of mediastinum	6	2	1 (1)
Carcinoid	1	—	—
Pancreatic carcinoma	4	3	—
Thyroid carcinoma	7	5	2 (1+, 3+)
Unknown primary	14	7	0
Ovarian carcinoma	1	—	—
Melanoma	10	6	0
Total	205	118	21

*Category I (A, B, C) responses evaluated according to Karnofsky et al (6).

TABLE 7.—Adriamycin in combination with vincristine, methotrexate with citrovorum rescue, and actinomycin D in adults with sarcomas

Disease	Total No. of patients	No. of evaluable patients	No. of responses* (duration in mos)
Leiomyosarcoma	3	3	2
Liposarcoma	5	4	3 (1+-4+)
Fibrosarcoma	1	0	0
Rhabdomyosarcoma	1	1	—
Spindle cell	3	1	—
Synovial	3	2	—
Ewing's	1	1	1 (6)
Osteogenic	5	4	2 (3, 4+)
Malignant schwannoma	1	—	—
Miscellaneous	1	—	—
Total	24	16	8

*Category I (A, B, C) responses evaluated according to Karnofsky et al (6).

TABLE 8.—Adriamycin in combination (protocol L10) in adults with acute lymphoblastic leukemia

		No. of patients with—	
	Total No. of patients	Complete remission	Partial remission
No prior treatment	16	13	2
Prior treatment	5	1	2

TABLE 9.—Adriamycin combined with bleomycin, cyclophosphamide, vincristine, and prednisone in adults with non-Hodgkin's lymphoma

Disease	Total No. of patients	No. of evaluable patients	No. of responses* (duration in mos)
Histiocytic	13	10	7 (2 CR†)
Lymphocytic	3	3	1

*Category I (A, B, C) responses evaluated according to Karnofsky et al (6).
† CR = complete remission.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053; dactinomycin
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
Bleomycin: NSC-125066
Citrovorum factor: NSC-3590; CAS reg. No. 6035-86-5; glutamic acid, *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8
Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,-11,20-trione, 17,21-dihydroxy-
Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); Oncovin

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Adriamycin (NSC-123127) in Pediatric Malignancies[1,2]

Charlotte Tan, Gerald Rosen, Fereshteh Ghavimi, Mahroo Haghbin, Lawrence Helson, Norma Wollner, and M. Lois Murphy[3,4]

SUMMARY

Five and one-half years of experience in the use of adriamycin in the treatment of pediatric malignancies are summarized. The dose schedule preferred is 20-30 mg/m^2/day for 2 or 3 consecutive days for sensitive tumors and 45 mg/m^2 for 2 consecutive days for resistant tumors. Side effects consisted of hematologic depression, alopecia, oral ulcers, nausea and vomiting, and occasionally an allergic reaction at the injection site. Extreme care to avoid drug extravasation is emphasized. In patients who had previously been treated with chemotherapy, adriamycin alone produced an overall complete and partial remission rate of 40% in acute leukemia and 30% in metastatic solid tumors. Adriamycin has activity in a variety of solid tumors. The median duration of remission in solid tumors with adriamycin alone is 3½ months. Longer remissions have been achieved when adriamycin is used in combination with other anticancer drugs. Adjuvant combination chemotherapy including adriamycin given in addition to surgery and/or radiation therapy has improved the survival of patients with embryonal rhabdomyosarcoma, Ewing's sarcoma, ovarian tumor, and osteogenic sarcoma.

[Cancer Chemother Rep Part 3, vol 6:259-266, 1975]

In 1964, through Prof. Di Marco et al (1), Dr. Karnofsky introduced daunorubicin to the Memorial Sloan-Kettering Cancer Center for clinical trials. An initial trial undertaken at the NCI in Milan in 20 adult patients with carcinoma had shown no therapeutic effect (2). Our initial study of daunorubicin (3) was in children because of the limited amount of drug available. Daunorubicin was found to be effective in producing remissions in leukemia and some cases of neuroblastoma, reticulum-cell sarcoma, and embryonal rhabdomyosarcoma.

At the Second Daunomycin Symposium in Rio de Janiero, Brazil, in October 1968, Prof. Di Marco mentioned a new antitumor antibiotic, adriamycin, which was structurally similar to daunorubicin except for the substitution of a hydrogen atom by a hydroxyl group in the acetyl radical (4). The initial clinical trials of adriamycin were begun at the NCI in Milan (5).

MATERIALS AND METHODS

Our clinical studies were begun in May 1969, and the data have been reported (6-8). This report summarizes our 5½ years of experience with adriamycin used in children with neoplastic disease, and presents a brief summary of the total adriamycin experience in children from other centers (especially of major study groups).

In general, a phase I drug is usually first studied in patients with solid tumors, but because of our results with daunorubicin, the first child who received adriamycin was a patient with acute leukemia in whom a remission was obtained. As of November 1974 at this center, 460 children with leukemia and lymphoma or solid tumors have received adriamycin either alone (137 patients) or in combination with other anticancer drugs (323 patients).

Dosage

We have previously reported the six dose schedules of adriamycin studied in children. These were daily single doses of 15 or 30 mg/m^2 for two or six doses/course, given consecutively or intermittently. A fifth dose schedule was 7.5-15 mg/m^2 every 6 hours for four doses and the last schedule was 60 mg/m^2 given in one single dose. Courses are repeated every 2-3 weeks. Most of our patients received the drug for 2-3 consecutive days. No advantage was observed in the other dose schedules. Our current dose for sensitive tumors, or if the drug is used as adjuvant therapy, is 20 mg/m^2/day for 3 consecutive days or 30 mg/m^2/day for 2 consecutive days. In resistant tumors, such as osteogenic sarcoma, most of the patients are given 45 mg/m^2/day for 2 consecutive days.

[1]Supported in part by Public Health Service grants CA-05826 and CA-08748 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[2]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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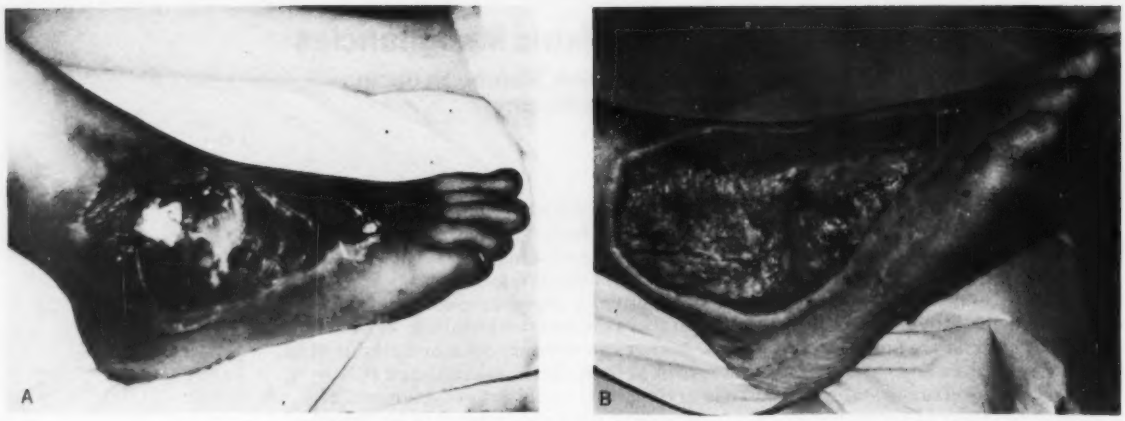

FIGURE 1.—Extravasation of adriamycin causing ulceration and necrosis at injection site and resulting in skin grafting. A = Before skin graft; B = after skin graft.

TOXIC EFFECTS

The toxic effects of adriamycin consist of hematologic depression, alopecia, oral ulcers, and gastrointestinal disturbances. In addition, one of the major side effects which needs to be emphasized is the severe local reaction, with phlebitis and local cellulitis followed by necrosis and fibrosis, resulting from extravasation of adriamycin. Therefore, extreme care must be employed in the intravenous (iv) injection of adriamycin, especially in children, who often have small and difficult veins.

Patient No. 1, a 14-year-old boy with osteogenic sarcoma who was treated on the T4 protocol, was given his usual iv dose of adriamycin at 45 mg/m^2 or 80 mg diluted with 40 ml of normal saline. The following day he exhibited blisters over the left foot at the injection site, followed by ulceration and necrosis. Debridement of the ulcer was followed by a split-thickness skin graft. It took 4 months for complete healing. During this period all other chemotherapy was withheld except cyclophosphamide. Figure 1 shows the ulceration before and after the skin graft.

Local rash, urticaria, and pain occurred occasionally at the injection site. Potentiation of the skin reaction in the irradiated area was observed in about 20% of the patients. Skin pigmentation, usually in the antiflexor areas of joints of hands and feet, has been observed in children with dark complexions, especially blacks or Orientals.

The incidence of adriamycin cardiomyopathy in our series is 16% and the details will be reported elsewhere. Gilladoga et al (9) reported the association of a higher incidence of adriamycin cardiomyopathy in children who also received irradiation to the mediastinum compared to those children who received adriamycin alone.

RESULTS

Leukemias

In previously treated patients with acute leukemia, adriamycin produced a complete remission rate of 15% and a partial remission rate of 30%. This is similar to our previous experience with daunorubicin in a comparable group of patients. In previously untreated patients (10) adriamycin was used in combination with prednisone, vincristine, and intrathecal methotrexate for induction; the remission rate was 99%. In the acute nonlymphoblastic leukemias, cytosine arabinoside and 6-thioguanine were used as induction, followed by adriamycin, and then later L-asparaginase as consolidation therapy. In both of these combination protocols, adriamycin was used as part of the multiple-drug regimen for maintenance of remission.

Solid Tumors

In previously treated patients who had metastatic disease, the overall response rate categories I-A, B, and C (complete and partial) was about 30%. With adriamycin alone, the duration of remission was usually short (a median of 3.5 months) but there were exceptions (table 1). The responsive tumors were Hodgkin's and non-Hodgkin's lymphomas, embryonal rhabdomyosarcoma, Ewing's sarcoma, and Wilms' tumor. The less commonly seen tumors, such as ovarian tumors, embryonal carcinomas, and malignant teratomas, were also sensitive to the effect of adriamycin.

TABLE 1.—Adriamycin used alone in solid tumors in children

Tumor type	Total No. of patients— Treated	Evaluable	No. of responses (mos) in categories— I-A,B,C	II	III
Hodgkin's disease	11	9	4 (1½-3)		
Non-Hodgkin's disease	6	4	1 (6¾)		
Embryonal rhabdomyosarcoma	14	12	4 (3-8)	1 (9)	
Neuroblastoma	12	10	1 (2)		
Ewing's sarcoma	8	8	3 (1,3½, 26½)		1 (7¾)
Wilms' tumor	6	6	3 (2,3½, 10)	1 (2)	
Ovarian teratoma	4	3	3 (4½,5, 14)		
Osteogenic sarcoma	4	2	0	1 (3)	
Hepatoma	3	3	0		
Embryonal carcinoma	2	2	1 (44+)		
Malignant teratoma (sacral)	1	1	1 (1)		
Miscellaneous	3	3	0		
Totals	74	64	21	3	1

One boy who was diagnosed in August 1970 as having embryonal carcinoma of the transverse mesocolon, showed a large inoperable necrotic and gangrenous tumor at exploratory laparotomy. In addition, he had biopsy-proven metastases to the left cervical nodes, associated with fever and leg pain. Adriamycin was started in November 1970 and he received a total of 1085 mg/m^2 in 16 courses over a period of 2 years. After his first course of adriamycin, he began to show signs of improvement followed by complete tumor regression. A second-look exploratory laparotomy was done on August 16, 1973, and there was no evidence of tumor. He now has no evidence of disease 4½ years after diagnosis and 2½ years after adriamycin was discontinued.

Soon after the observation that adriamycin was effective in a wide variety of solid tumors in children, a multidisciplinary protocol (T2) was designed for all childhood tumors (fig 2). This consisted of surgery (with removal of the primary tumor) and/or irradiation for residual disease, with simultaneous administration of a course of actinomycin D, followed by two courses of adriamycin, and finally vincristine together with cyclophosphamide. There was a rest period of usually 2 weeks for hematologic recovery between each phase of chemotherapy. Recently, in order to limit the total cumulative dose of adriamycin, this protocol was modified by giving only one course of adriamycin instead of two courses per cycle after the first two cycles. The total chemotherapy course lasts for approximately 20 months. Table 2 shows the results of the T2 protocol in 138 children with solid tumors.

In previously treated patients with metastatic

TABLE 2.—Solid tumors in children—multidisciplinary protocol T2

Tumor type	Total No. of patients— Treated	Evaluated	No. of responses (mos) in categories— I-A,B,C	II	III
Embryonal rhabdomyosarcoma	55	53	14 (2-43+)	1 (3)	32(1+-45+)
Ewing's sarcoma	30	27	7(1+-19+)		20(2+-52+)
Neuroblastoma	14	11	4 (3-6¾)	2 (4,12)	1 (10)
Ovarian tumor	13	12	10(1+-30)	1 (5)	1 (11+)
Wilms' tumor	4	4	2 (3,6¾)		1 (9+)
Liver tumor	3	2	1 (6)		
Malignant teratoma (sacral)	3	3	1(9+)		1 (40+)
Synovial sarcoma	2	2	1 (26+)		
Angiosarcoma	1	1	1 (14)		
Pulmonary blastoma	1	1		1 (6)	
Lymphoepithelial carcinoma	1	1		1 (4)	
Miscellaneous tumors	11	9			5 (2-29+)
Totals	138	126	41	6	61

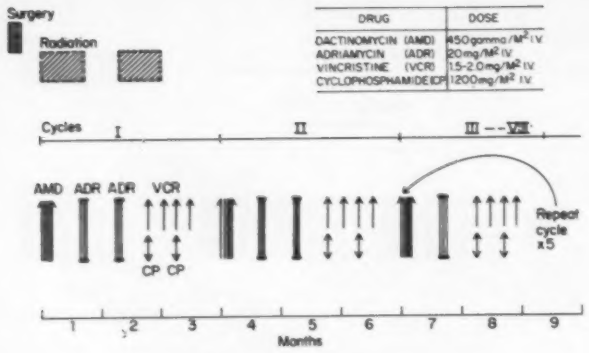

FIGURE 2.—Multidisciplinary protocol (T2) for the management of childhood solid tumors.

disease, the overall complete and partial remission rate was again about 30%. However, the median duration of remission for these patients on the T2 protocol was 8 months, a twofold increase compared to that of adriamycin alone. A group of children with ovarian tumors consisting of malignant teratoma, embryonal carcinoma, and endodermal sinus tumor all had tumor response with a median duration of 14 months. Six children with metastatic disease showed no evidence of progression of disease (category II) for 3-12 months while on the T2 protocol. This may be due to the effect of chemotherapy. For those patients who had their primary and/or metastatic disease treated by surgical removal or radiotherapy and simultaneous adjuvant chemotherapy (category III), the results were especially impressive in embryonal rhabdomyosarcoma and Ewing's sarcoma. Ghavimi et al (11) reported the results of this multidisciplinary treatment in 29 children with embryonal rhabdomyosarcoma and showed an 81% actuarial survival at 2 and 3 years. An additional 20 children were enrolled on this protocol and the actuarial survival remained 80%. Of these 49 previously untreated patients, 16 were staged I-A, indicating that the tumor was localized and completely resected, the regional nodes were not involved, and the margins were clear microscopically. None of these 16 children received radiotherapy and in all cases the only chemotherapy given was that included in the T2 protocol. They have shown no evidence of disease for 4-46 months.

Rosen et al (12) reported an improved disease-free survival in children with Ewing's sarcoma treated with radiation therapy and adjuvant chemotherapy. Of a total of 16 children on the T2 protocol, five have relapsed after 18-38 months. These included the three patients who had metastases at diagnoses which were treated and cleared at the start of the T2 protocol.

Another multiple-drug protocol was designed for the treatment of advanced Hodgkin's disease in children (13). It uses adriamycin as the initial drug followed 2 weeks later by prednisone and procarbazine orally, three or four weekly doses of vincristine, and then an iv dose of cyclophosphamide at 40 mg/kg. After 3 weeks of rest, the chemotherapy cycle is repeated and continued, in most of the patients for a period of 20 months. Of 25 patients with stage III and IV Hodgkin's disease who had adequate treatment on this multiple-drug protocol, all achieved remission; 20 were complete and five were partial. The median duration was 18+ months for complete remissions and 3+ months for partial remissions. Eighteen of the 20 patients who had complete remission remained free from disease (two for less than 1 year, 16 for 1-5+ years) after the start of the multiple-drug regimen.

5-Azacytidine was reported to have produced remission in acute leukemia (14,15); however, the major side effect was severe nausea and vomiting. In our initial studies with 5-azacytidine alone (16), only two of the 12 children with acute leukemia achieved transient partial remissions.

The combination of 5-azacytidine and adriamycin was then studied using 5-azacytidine at 100 mg/m² twice a day and a dose of adriamycin at 7.5 mg/m² given with the morning dose of 5-azacytidine for a total of 5 days. The nadir of hematologic depression usually occurred within 2 weeks and recovered by the third week. This is similar to that of adriamycin alone. In some patients, five daily iv doses of bleomycin at 0.25 mg/kg were given as continuing chemotherapy during the period of hematologic depression. Table 3 shows the complete and partial remissions in five of the eight patients with acute leukemia, all of whom had previous chemotherapy (including some who had adriamycin or 5-azacytidine alone). In four children with malignant liver tumors (hepatoblastoma, three; hepatocellular carcinoma, one), two achieved complete tumor regression for 5½ and 14 months respectively and another patient for less than 1 month.

For resistant tumors, Rosen et al (17, 18) reported the use of adriamycin sequentially with high-dose methotrexate and citrovorum factor rescue in advanced metastatic osteogenic sarcoma (T3 protocol). In patients with far-advanced and progressive disease, optimal chemotherapy every 2 weeks seemed to be necessary. In order to limit the cumulative total dose of adriamycin to around 600 mg/m², thereby avoiding cardiac toxicity, the T4 protocol was designed using vincristine prior to the high-dose methotrexate and citrovorum factor rescue. Two weeks after methotrexate, a dose of cyclophosphamide was given at 40-60 mg/kg; 2 weeks later two doses of adriamycin were given at 45 mg/m²/day, and then another dose of cyclophosphamide. This cycle was then repeated. Table 4 shows the results of adriamycin in these combination protocols in resistant childhood tumors. The overall response rate in patients with advanced metastatic disease was 55% with a median duration of remission varying from 1 to 17 months.

TABLE 3.—Adriamycin and 5-azacytidine (followed by bleomycin) in children

Tumor type	Total No. of patients— Treated	Total No. of patients— Evaluable	No. of remissions (mos) Complete	No. of remissions (mos) Partial	No. of responses (mos) in category I-A,B,C
Acute lymphoblastic leukemia	9	8	1(4)	1 (¼)	
Acute myeloblastic leukemia	2	2	0	2 (1½, 2½)	
Erythroleukemia	1	1	0	1 (1)	
Liver tumor	4	4			2 (5½, 14)
Embryonal adenocarcinoma	2	1			1 (2)
Neuroblastoma	2	1			0
Lymphoma	2	0			
Alveolar soft part sarcoma	1	1			0

TABLE 4.—Adriamycin in combination in resistant tumors in children

Protocol No. and tumor type	Total No. of patients— Treated	Total No. of patients— Evaluated	No. of responses (mos) in categories— I-A,B,C	II	III
T3					
Osteogenic sarcoma	14	14	7 (1-6)	2 (3,4)	1 (4)
Synovial sarcoma	1	1	1 (17)		
Lymphoepithelial carcinoma	1	1	1 (5)		
T4					
Osteogenic sarcoma	36	30	9 (1¾-13)	4 (4-23+)	12 (4+-22+)
Synovial sarcoma	2	2	1 (5)		1 (10+)
Chondrosarcoma	2	2			1 (5+)
Malignant hemangiopericytoma	1	1			1 (6+)

TOTAL ADRIAMYCIN EXPERIENCE OF MAJOR CHILDREN'S COOPERATIVE STUDY GROUPS

Tables 5-7 include data obtained from the following study groups: Southwest Oncology Group (SWOG); Memorial Sloan-Kettering Cancer Center (MSKCC); Roswell Park Memorial Institute (RPMI); Acute Leukemia Group B (ALGB); NCI Milan; St. Jude Children's Research Hospital (St. Jude); Children's Cancer Study Group (CCSG); M.D. Anderson Hospital and Tumor Institute (M.D. Anderson); and Children's Hospital of Stanford (CH Stanford).

Table 5 summarizes the experiences with adriamycin in the treatment of acute lymphoblastic and nonlymphoblastic leukemias of the SWOG (19),[5] MSKCC (6-8), and RPMI (20). The average overall complete and partial remission for acute lymphoblastic or nonlymphoblastic leukemia was about 40%. Table 6 shows the overall response rates of adriamycin in childhood solid tumors of the various study groups (8,20-25). In the responsive tumors, including lymphoma, Wilms' tumor, neuroblastoma, embryonal rhabdomyosarcoma, and Ewing's sarcoma, the remission rate varied from 40% to 67%. In osteogenic sarcoma, the overall response rate among all study groups was 22%.

Table 7 summarizes adriamycin used as adjuvant therapy in combination with other drugs in primary osteogenic sarcoma. In a study by the ALGB (26) using adriamycin alone (with observations up to 38.5 months), 11 of 44 patients have relapsed. At 18 months, 60% of the patients were expected to be free from evidence of disease. Sutow (27) reported studies from M.D. Anderson in a protocol called "Conpadri" (as an adjuvant to amputation) using adriamycin in a four-drug regimen along with vincristine, cyclo-

[5]Starling KA, Berry DH, Britton HA, et al. Three dose regimen of adriamycin for induction of remission in acute leukemia in children. In preparation, 1975.

TABLE 5.—Adriamycin in childhood acute leukemia

Study group	Ref No.	Lymphoblastic			Nonlymphoblastic		
		No. of patients—		Response rate (%)	No. of patients—		Response rate (%)
		Treated	With CR + PR*		Treated	With CR + PR*	
SWOG	19 (footnote 5)	41	22	54	9	5	56
SWOG	19 (footnote 5)	122	64	52	28	9	32
MSKCC	6-8	50	21	42	6	3	50
RPMI	20	30	6	20	—	—	—

*CR = complete response, PR = partial response.

TABLE 6.—Adriamycin in childhood tumors

Diagnosis	Study group (ref No.)						
	ALGB (21)	SWOG (22)	MSKCC (8)	NCI Milan (23)	St. Jude (24)	CCSG (25)	RPMI (20)
	(No. of patients with CR or PR/No. of patients evaluable)						
Lymphoma	2/9	4/8	5/13	5/7	2/3		1/2
Wilms' tumor	11/17	7/13	4/6	5/5	4/4	0/1	
Neuroblastoma	14/40	9/18	1/10	9/11	3/6	2/13	5/8
Embryonal rhabdomyosarcoma	7/17	4/11	5/12	1/1	2/6	3/9	0/1
Ewing's sarcoma	6/11	1/6	3/7	2/2	0/2	1/3	2/3
Osteosarcoma	4/27	2/12	1/2	0/3	5/7		1/7
Other sarcoma	10/17	1/7		5/7	0/2		0/2
Ovarian carcinoma	1/2		3/3				
Adenocarcinoma		1/1	1/2				
Hepatoma	2/3	1/2	0/3	1/2	1/1		
Malignant teratoma			1/1				0/2
Retinoblastoma		2/6	0/1				
Miscellaneous		0/4	0/2	2/2	0/4	0/1	

TABLE 7.—Adriamycin in primary osteosarcoma

Study group	Ref No.	Drug*	Total No. of patients	Disease-free patients (%)	Median duration of response (mos)
ALGB	26	A	44	60	10
M. D. Anderson	27	Conpadri	43	55	36,19,7
SWOG	27	Compadri II	24	73	14
MSKCC	18	VCR → MTX + CF → CY → A → CY	23	90	10
St. Jude	28	A → CY → MTX + CF	16	87	8
CH Stanford	29	MTX + CF → VCR + A → VCR + CY	15	80	14

*A = adriamycin; VCR = vincristine; MTX = methotrexate; CF = citrovorum factor; CY = cyclophosphamide.

phosphamide, and melphalan. Of 43 patients treated, 24 (55%) remained free from disease 2-49 months after amputation. The median duration was 36 months for the first group and 7 months for the most recent group. A subsequent study by the SWOG was done with "Compadri II," by adding high-dose methotrexate and citrovorum factor rescue. In a group of 24 patients, 70% were disease free at 12 months. At MSKCC using the T4 protocol, 21 of 23 patients (90%) remained free from disease with a median duration of 10+ months. In studies at St. Jude (28) using adriamycin, cyclophosphamide, high-dose methotrexate, and citrovorum factor rescue, 14 of 16 patients (87%) remained disease free with a median duration of 8+ months. At CH Stanford (29) methotrexate and citrovorum factor rescue were used, followed by vincristine, adriamycin, and then vincristine and cyclophosphamide; 12 of 15 patients (80%) remained disease free with a median duration of 14+ months.

DISCUSSION

The dose schedule of adriamycin used at MSKCC has been 20-30 mg/m^2/day for 2 or 3 consecutive days for sensitive tumors and 45 mg/m^2/day for 2 consecutive days for resistant tumors. In our experience, higher single doses caused more frequent nausea and vomiting. With these schedules a course can be repeated every 2 weeks with minimal hematologic depression. Stomatitis was very infrequent. Potentiation of a skin reaction in the irradiated area occurred in about 20% of the patients when the drug was given simultaneously with radiation therapy. However, it was of mild degree compared to that of actinomycin D. With actinomycin D, skin reaction over the previously irradiated area occurred in 60% of the patients.

Skin pigmentation was reported after the administration of adriamycin (30-32). Some of these patients also received other drugs. Symmetrical brown pigmentation, most marked on the extensor surfaces of the joints of fingers and toes, was observed in patients after prolonged therapy with NSC-7364 (33) and in patients treated with folic acid antagonists (34).

In children with acute leukemia, both lymphoblastic and nonlymphoblastic, the overall remission rate of adriamycin is similar to that of daunorubicin although these were not randomized studies. Evans et al (25) reported a randomized study of daunorubicin and adriamycin in the treatment of late-stage childhood solid tumors, and found there was no difference in the response rate of patients with neuroblastoma. In our initial report of daunorubicin, six of 15 children with neuroblastoma had complete or partial responses. Of these 15, however, four had had no prior therapy and another six had had their disease for less than 6 months prior to daunorubicin therapy. These factors most likely contributed to the higher response rate of that group. In our study with adriamycin alone, five of ten children with neuroblastoma had had daunorubicin previously. In the other five, only one had tumor regression which lasted for 2 months. In the T2 protocol, with three other anticancer agents added, four of 11 had complete or partial responses. However, Helson et al (35) concluded that the T2 protocol with four drugs was of minimal additional benefit to these patients' survival time when compared with the previous group receiving three drugs (vincristine, cyclophosphamide, and daunorubicin).

In other types of childhood metastatic tumors, adriamycin used in combination in the T2 protocol did not increase the response rate over that of adriamycin used alone, but the duration of remission was increased twofold. With adjuvant chemotherapy in previously untreated patients, the disease-free survivals in both embryonal rhabdomyosarcoma and Ewing's sarcoma were definitely increased over the previous treatment regimens. Children with ovarian tumors have responded favorably to adriamycin. In children with far-advanced Hodgkin's disease, the duration of remission for single drugs, such as adriamycin, procarbazine, or vinblastine, was 2 months. With adriamycin followed by other effective drugs in Hodgkin's disease (13), the duration of complete remission was 18+ months. At 4 years, the actual survival of patients treated with the multiple-drug protocol was 60% versus 30% in earlier studies with a single drug. The effect of adriamycin alone in children with metastatic hepatoma was disappointing although the numbers were very few. Olweny et al (36) reported that all of their 11 evaluable adult patients with primary hepatocellular carcinoma treated with adriamycin alone responded, with three complete tumor regressions for 3, 6, and 7 months respectively. The median survival time of the evaluable patients was 8 months.

In the combination of 5-azacytidine and adriamycin, the total dose of adriamycin per course was only 37.5 mg/m^2, and there was tumor regression in three of the four children with hepatoma. In contrast, with adriamycin used alone at 60-90 mg/m^2, none of the three hepatoma patients responded although this number is not large enough to be significant.

The study of the ALGB with adriamycin used alone after surgery in the treatment of osteogenic sarcoma, indicated the effectiveness of this drug. However, the trend is to use adriamycin in combination with other effective drugs for prolonged control in resistant tumors. In patients with far-advanced and progressive disease, optimum chemotherapy every 2-3 weeks seemed necessary. Therefore, combining adriamycin with other effective anticancer drugs should not only add to the therapeutic effect and decrease the frequency of adriamycin administration to avoid cumulative cardiac toxicity, but should also possibly decrease the rapidity of drug resistance.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053; dactinomycin
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
L-Asparaginase: NSC-109229; CAS reg. No. 9015-68-3
5-Azacytidine: NSC-102816; CAS reg. No. 320-67-2; *s*-triazin-2-(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-
Bleomycin: NSC-125066
Citrovorum factor: NSC-3590; CAS reg. No. 6035-86-5; glutamic acid, *N*-[*p*-[[(2-amino-5-formyl-5, 6, 7, 8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-, calcium salt
Conpadri=cyclophosphamide, vincristine (Oncovin), melphalan, and adriamycin
Compadri II=cyclophosphamide, vincristine (Oncovin), methotrexate, citrovorum factor, melphalan, and adriamycin
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,-2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6; daunomycin
Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-; L-phenylalanine mustard
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
NSC-7364: CAS reg. No. 7761-45-7; pyrimidine, 2,4-diamino-5-(3,4-dichlorophenyl)-6-methyl-
Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-
Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride
6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-
Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate; Velban
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); Oncovin

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Phase II Study of Adriamycin (NSC-123127) in Childhood Solid Tumors[1,2]

Jaw J. Wang,[3] James F. Holland,[4] and Lucius F. Sinks[3,5,6]

SUMMARY

The Acute Leukemia Group B undertook a phase II study to determine the effect of adriamycin in children with disseminated neoplasia. Among 145 patients evaluated, objective response was seen in 11 of 17 (68%) patients with Wilms' tumor, 14 of 40 (35%) with neuroblastoma, six of 11 (55%) with Ewing's sarcoma, seven of 17 (41%) with rhabdomyosarcoma, four of 27 (15%) with osteosarcoma, and two of three (66%) with hepatoblastoma. All patients received prior chemotherapy except for those who had osteogenic sarcomas. The major toxic effects of adriamycin included leukopenia, thrombocytopenia, marrow hypoplasia, stomatitis, vomiting, and alopecia. Two patients demonstrated adriamycin cardiomyopathy and congestive heart failure. The activity of adriamycin in these patients compared favorably with that of actinomycin D, vincristine, and cyclophosphamide. This supports the use of adriamycin in combination with standard agents in patients earlier in their therapeutic course.

[Cancer Chemother Rep Part 3, vol 6:267-270, 1975]

Adriamycin, a cytotoxic antibiotic derived from *Streptomyces peucetius* var. *caesius*, has been found to be active in a variety of neoplastic diseases (1,2). The initial clinical trials of adriamycin were begun at the NCI in Milan, Italy. Bonadonna et al (3) and Monfardini et al (4) reported its use in a total of 155 patients (34 children and 121 adults).

Phase I studies with adriamycin were conducted at Roswell Park Memorial Institute (5). The tolerated dose was 20 mg/m^2/day × 4 intravenously (iv) or 30 mg/m^2/day × 3 iv repeated every 3 weeks. There were regressions in children with Ewing's sarcoma, neuroblastoma, rhabdomyosarcoma, and osteogenic sarcoma.

In April of 1971, the ALGB activated a phase II study to determine the effect of adriamycin given at 3-week intervals to children with disseminated neoplasia.

METHODS

The criteria for entry into this study included *(a)* a histologically proven diagnosis of malignant neoplasm refractory to standard treatment, *(b)* a maximum age limit of 20 years, and *(c)* a minimum leukocyte count of 4000 cells/mm^3, platelet count of 100,000 platelets/mm^3, and blood urea nitrogen less than 25 mg/100 ml.

Initially, patients were randomized to one of two different doses of adriamycin. Adriamycin was given at a dose of either 15 or 20 mg/m^2/day × 4 by iv push every 3 weeks. The 15-mg/m^2/day × 4 iv schedule was later replaced by 30 mg/m^2/day × 3 by iv push every 3 weeks. The total maximal cumulative dose of adriamycin was limited to 500 mg/m^2. A leukocyte count of less than 1000 cells/mm^3 required a downward adjustment of succeeding doses of adriamycin to 75% of the initial dose.

A complete remission was defined as the disappearance of all clinical evidence of tumor persisting for a minimum of 4 weeks. A partial remission was defined as a 50% or greater decrease in the product of the two largest perpendicular diameters of all measurable lesions persisting for a minimum of 4 weeks without a simultaneous increase in the size of any lesion or the appearance of any new lesion. Improvement was defined as a 25%-50% decrease in the product of the two largest perpendicular diameters of any measurable lesion lasting for a minimum of 4 weeks, or a greater than 50% decrease lasting less

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[1]Adriamycin: CAS reg. No. 23214-92-8. Supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute.

[2]This study was done by the Acute Leukemia Group B (ALGB); *James F. Holland*, chairman.

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[5]The authors are grateful to Mr. Oliver Glidewell, ALGB statistician, for his expert statistical analysis and to the following investigators for entering patients into the study: *Drs. Geoffrey Falkson* (University of Pretoria, South Africa), *Frederick Ruymann* (Walter Reed Army Medical Center), *Faith Kung* (University of California at San Diego), *Richard Patterson* (Bowman Gray School of Medicine), *Barbara Jones* (West Virginia University Medical Center), *Harold Maurer* (Medical College of Virginia), *Omer Burgert* (Mayo Clinic), and *Jurg Sartorius* (University Children's Hospital, Basel, Switzerland).

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than 4 weeks without a simultaneous increase in the size of any lesion or the appearance of any new lesion. Patients who did not meet the above criteria for complete or partial remission or improvement were classified as "no response."

RESULTS

A total of 171 children were entered into the phase II study. Eleven patients were ineligible for evaluation either because they were entered into the study too late or sufficient data were not reported, and 15 patients were ineligible because of miscellaneous protocol errors. The remaining 145 patients contributed the data for the basis of this report.

Therapeutic Responses

The overall response rate was 39% (57 of 145). The best results were noted in Wilms' tumors, 68% (11 of 17). Other remissions included neuroblastoma, 14 of 40; Ewing's sarcoma, six of 11; lymphomas, two of nine; osteosarcoma, four of 27; rhabdomyosarcoma, seven of 17; hepatoblastoma, two of three; synovial sarcoma and other miscellaneous tumors, ten of 17 (table 1).

The response in Wilms' tumor is most interesting. Three patients have had complete remissions and eight have had partial remissions. The three patients with complete remissions have a median length of remission of 10 months and the eight patients with partial remissions have a median remission duration of 7 months.

Tumor response is compared with dosage in table 2. Objective responses were noted in 29% (seven of 24) of the patients who received 15 mg/m^2/day × 4, 38% (26 of 69) of the patients who received 20 mg/m^2/day × 4, and 46% (24 of 52) of the patients who received 30 mg/m^2/day × 3.

Figure 1 compares the survival for responders (complete and partial response) and nonresponders for the total group of 145 patients. The graph shows a statistically significant difference at least at the 0.05 level by Breslow's test of censored observation.

Adriamycin Toxicity

Table 3 summarizes the hematopoietic toxic effects of adriamycin. Severe leukopenia (degree 3-4) was observed in 30% (44 of 145) of the patients. Severe thrombocytopenia was less common but was observed in 12% (18 of 145) of the patients. Other toxic effects included nausea and vomiting, mucositis, anorexia, and alopecia.

Two patients experienced congestive heart failure at a total cumulative dose of 500 and 450 mg/m^2, respectively. Both patients had Wilms' tumor and had received prior irradiation to the chest including the mediastinum. Both patients had transient improvement after digitalization but subsequently died of recurrent disease.

DISCUSSION

In this study, we confirmed the previously reported antitumor effect of adriamycin in several important childhood solid tumors, including Wilms' tumors,

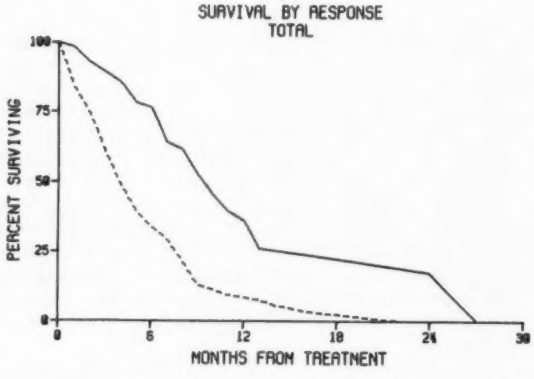

FIGURE 1.—Comparison of survival for responders (——) and non-responders (- - - -) for the total patient group; No. of deaths/No. of entries = 35/57 and 73/88 for responders and non-responders, respectively.

TABLE 1.—Results by diagnosis*

Diagnosis	Adriamycin dose (mg) 60	80	90	Totals
Neuroblastoma	2/7	8/24	4/9	14/40
Wilms' tumor	2/4	6/9	3/4	11/17
Ewing's sarcoma	0	3/6	3/5	6/11
Lymphoma	0/4	2/4	0/1	2/9
Osteosarcoma	1/6	1/12	2/9	4/27
Rhabdomyosarcoma	0/1	3/8	4/8	7/17
Other	2/2	3/6	7/12	12/20

*Includes total complete and partial responses/total No. of patients (four patients without diagnoses are not reported).

TABLE 2.—Results of therapy

Adriamycin dose (mg/m²)	Total No. of evaluable patients	Response type*				
		CR	PR	IMP	NR	Total CR + PR (%)
60	24	4	3	12	5	29
80	69	5	21	28	15	38
90	52	8	16	18	10	46
Totals	145	17	40	58	30	39

*CR = complete response; PR = partial response; IMP = improvement; NR = no response.

TABLE 3.—Adriamycin toxicity

Adriamycin dose (mg/m²)	No. of patients with—*											
	Low wbc count†				Low platelet count				Infection			
	0	1-2	3	4	0	1-2	3	4	0	1-2	3	4
60	5	13	5	0	11	6	2	0	13	1	1	0
80	14	30	15	7	43	13	5	3	30	2	2	3
90	4	29	12	5	23	18	6	2	16	3	2	0

*Hematopoietic toxicity was defined as follows:

Degree	*Wbc (cells/mm³) count*	*Platelet (platelets/mm³) count*
0	>4000	>100,000
1	4000-3000	100,000-75,000
2	2999-2000	74,500-50,000
3	1999-1000	49,500-25,000
4	<1000	<25,000

Infection was defined as follows:

Degree	*Definition*
0	None
1-2	Mild to moderate
3	Severe
4	Life-threatening

TABLE 4.—Comparison of activity of adriamycin with activity of other drugs against solid tumors in children

Drug	No. of patients—	
	Evaluated	Responding (%)
	Wilms' tumor	
Actinomycin D	26	10 (39)
Vincristine	31	23 (74)
Adriamycin	17	11 (65)
	Ewing's sarcoma	
Cyclophosphamide	21	13 (62)
Vincristine	8	4 (50)
Adriamycin	11	6 (55)
	Neuroblastoma	
Cyclophosphamide	78	52 (67)
Vincristine	71	29 (40)
Adriamycin	14	40 (35)
	Rhabdomyosarcoma	
Actinomycin D	21	6 (29)
Vincristine	38	19 (50)
Cyclophosphamide	56	33 (59)
Adriamycin	17	7 (41)

Ewing's sarcomas, neuroblastomas, rhabdomyosarcomas, and osteogenic sarcomas. The effect of adriamycin on these tumors compared favorably to that of conventional drugs reported in the literature (actinomycin D, cyclophosphamide, vincristine)[7] as shown in table 4.

Although the maximum cumulative dose of adriamycin was limited to 500 mg/m^2 in this study, two patients with Wilms' tumor had congestive heart failure after 500 and 450 mg/m^2 of adriamycin, respectively. Since both patients had received prior radiation to the chest, additive toxicity between adriamycin and irradiation of the heart is a possible explanation. We would recommend that the total cumulative dose of adriamycin be limited to 400 mg/m^2 in those patients who have received or are to receive irradiation to the chest.

Hepatoblastoma is a tumor rarely seen in children. It is noteworthy that two patients had complete remissions after treatment with adriamycin suggesting that adriamycin might be the drug active in this tumor. Further study of the effect of adriamycin on this tumor is needed.

This study supports the use of adriamycin therapy in combination with other standard agents, as primary adjuvant therapy in childhood solid tumors.

[7]Actinomycin D: NSC-3053.

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

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Adriamycin (NSC-123127) Used Alone and in Combination for Soft Tissue and Bony Sarcomas[1,2,3]

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SUMMARY

During the past 5 years, the Southwest Oncology Group (SWOG) and the M.D. Anderson Hospital have been engaged in a series of protocols using adriamycin alone or in combination in an attempt to improve the chemotherapy available for adult patients with soft tissue and bony sarcomas. Our first study, however, involved another drug, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388), which produced a 17% response rate in 53 patients. Our next investigation was with adriamycin alone which produced a 31% response rate in 49 patients. Subsequently, we combined these two agents in nearly full doses, and in a large study of 218 patients we observed a 42% overall response rate including a 10% complete response rate. It was particularly gratifying that this last protocol produced statistically and clinically significant improvements in survival rates in responding patients, especially compared to our results seen with adriamycin alone. Twenty of 25 complete responders to the two-drug combination are still alive and responding nearly 3 years from the start of their chemotherapy. Our fourth study added vincristine to the above two-drug combination, but little improvement was observed with this addition except possibly in a few soft tissue sarcomas. Our next investigation included cyclophosphamide, vincristine, adriamycin, and NSC-45388, and with this regimen we observed in 136 patients 14% and 55% complete and overall response rates respectively, suggesting once again definite improvement. Currently, we are exploring the role of actinomycin D in these protocols as well as the use of a protected environment to further improve response rates. In addition, an adjuvant study utilizing combination chemotherapy with adriamycin together with radiotherapy and extirpative surgery is currently in progress for high-risk-for-recurrence, soft tissue sarcoma patients at the time of the original diagnosis. We feel that combination chemotherapy with adriamycin represents a significant advance in the therapeutic management of adult patients with metastatic sarcomas, and may offer more than palliation in some situations where it is used as an adjuvant.

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[2]Adriamycin: CAS reg. No. 23214-92-8. All drugs used in this study were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

[3]This study was done by the Southwest Oncology Group (SWOG) and the M.D. Anderson Hospital.

[4]The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute at Houston *(Drs. Gottlieb, Bodey, Rodriguez, Blumenschein, Burgess, Sinkovics, and Moon* [assistant biometrician, SWOG]); Wayne State University Medical School, Detroit, Mich *(Dr. Baker)*; Henry Ford Hospital, Detroit, Mich *(Dr. O'Bryan)*; University of Kansas Medical Center, Kansas City *(Dr. Hoogstraten,* chairman, SWOG); Southern Nevada Memorial Hospital, Las Vegas *(Dr. Quagliana)*; The Tumor Institute of the Swedish Hospital Medical Center, Seattle, Wash *(Dr. Rivkin)*; University of New Mexico School of Medicine, Albuquerque *(Dr. Saiki)*; Wilford Hall United States Air Force Medical Center, Lackland Air Force Base, San Antonio, Tex *(Dr. Coltman)*; The Diagnostic Clinic of Houston, Tex *(Dr. Sullivan)*; The University of Mississippi Medical Center, Jackson *(Dr. Thigpen)*; Oklahoma Medical Research Foundation, Oklahoma City *(Dr. Bottomley)*; Ohio State University Hospitals, Columbus *(Dr. Balcerzak).*

[5]The authors are deeply indebted to the Statistical Office of the SWOG and particularly Mrs. Dorothy Abernathy for their immense clerical efforts. We also appreciate the scientific advice of Drs. Emil J Freireich, Robert B. Livingston, Robert D. Lindberg, William O. Russell, and Edmund A. Gehan. Additional thanks are extended to Miss Helen Wall for secretarial assistance.

[6]*Reprint requests to:* SWOG Statistical Office, 6723 Bertner Dr., Houston, Tex 77025.

TABLE 1.—Completed protocols of the SWOG and the M.D. Anderson Hospital for metastatic sarcomas

Protocol No.	Period of administration	Drugs	Regimen used with— Adequate marrow reserve	Regimen used with— Inadequate marrow reserve
SWOG-434 (phase II)	1969-70	NSC-45388	250 mg/m²/day,Days 1-5	200 mg/m²/day,Days 1-5
SWOG-442 (phase II)	1971-72	Adriamycin	75 mg/m²	45-60 mg/m²
SWOG-7206 (dose response)	1973-74	Adriamycin	45-75 mg/m²	25-50 mg/m²
SWOG-445	1971-72	Adriamycin NSC-45388	60 mg/m²,Day 1 250 mg/m²/day,Days 1-5	45 mg/m²,Day 1 200 mg/m²/day,Days 1-5
SWOG-7210	1972-73	Vincristine Adriamycin NSC-45388	1.5 mg/m²(top dose 2 mg),Days 1,5 60 mg/m²,Day 1 250 mg/m²/day,Days 1-5	1.5 mg/m²(top dose 2 mg), Days 1,5 45 mg/m²,Day 1 200 mg/m²/day,Days 1-5
DT 73-02	1973-74	Cyclophosphamide Vincristine Adriamycin NSC-45388	500 mg/m²,Day 1(iv) 1.5 mg/m²(top dose 2 mg),Days 1,5 50 mg/m²,Day 1 250 mg/m²/day,Days 1-5	400 mg/m²,Day 1(iv) 1.5 mg/m²(top dose 2 mg), Days 1,5 40 mg/m²,Day 1 200 mg/m²/day,Days 1-5

*All protocols were repeated at 3-wk intervals if blood counts had recovered.

In 1969, when the Southwest Oncology Group (SWOG) and the M.D. Anderson Hospital became involved in the treatment of metastatic sarcomas in adults, the generally available chemotherapy was quite discouraging. Patients were usually treated with vincristine, cyclophosphamide, and/or actinomycin D in combination or in sequence.[7] Response rates for this type of therapy in adults never exceeded 20%, and only rarely were long-term responses reported, with most patients receiving little clinical benefit from their treatment (1). These results were particularly disheartening because of the rather optimistic observations that were being made with these same drugs used in children, predominately those with rhabdomyosarcoma (2).

Because of the paucity of effective drugs available, the SWOG decided to include patients with sarcomas in several of the new drug trials that were currently under evaluation, which included the chemically synthesized purine analog NSC-45388.[8] Treating patients who had frequently failed with the above chemotherapeutic agents, we observed one complete and eight partial remissions in 53 evaluable patients, for an overall response rate of 17%. While this was not extremely encouraging, considering the advanced nature of the patients entered into the study we felt we had at least uncovered an additional drug that could be offered to patients with metastatic sarcomas. The regimen we utilized was the SWOG protocol No. 434 as shown in table 1 (3).[9]

Our next study involved adriamycin. Using the regimen outlined in table 1 (SWOG-442), we treated 49 evaluable patients and observed two complete and 13 partial remissions for an overall response rate of 31%. These results suggested that adriamycin was the most effective single agent we had used in adult sarcoma patients (4).

After completion of this study, we were aware that several other cooperative groups and institutions were also exploring adriamycin's efficacy, and we knew of three complete and 33 partial remissions in 131 patients recorded in the entire literature, for an overall response rate of 27% (5). We have just recently reported a more up-to-date exhaustive literature review and this same overall response rate appears to be holding, as summarized in table 2 (6). For clarification purposes, in this table and in all of our results a response will indicate a 50% or greater decrease in the sum of the areas of all measurable metastatic lesions. Complete responses will indicate the complete disappearance of all measurable lesions as well as all symptoms due to the tumor. The minimum duration of these responses must be 1 month. Patients whose disease remains stable or whose lesions regress less than 50% are considered "no change" or "stable" patients, but will be classified as nonresponders in the tables included in this

[7]Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,-2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Actinomycin D: NSC-3053.

[8]NSC-45388: CAS reg. No. 4342-03-4; 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide; DIC.

[9]Luce JK. Results of phase II study of DIC in sarcomas. SWOG protocol No. 434. In preparation.

TABLE 2.—Single-drug therapy with adriamycin in metastatic sarcomas: cumulative literature survey*

Diagnosis	No. of patients	No. of responses	Response rate (%)
Soft tissue sarcomas			
Rhabdomyosarcoma	79	21	27
Fibrosarcoma and neurofibrosarcoma	38	9	24
Leiomyosarcoma	37	12	32
Synovial cell sarcoma	22	7	32
Liposarcoma	19	6	32
Angiosarcoma	16	7	44
Undifferentiated sarcoma	16	3	19
Unspecified and miscellaneous	130	31	24
Total	357	96	27
Bone sarcomas			
Osteogenic sarcoma	81	18	22
Ewing's sarcoma	63	27	37
Chondrosarcoma	17	1	6
Total	161	46	29
All sarcomas	518	142	27

*Modified from Gottlieb (ref 6).

paper. Patients with obvious progression are said to have "increasing disease" which will imply that their disease grew or new lesions appeared during drug treatment.

While it was clear that adriamycin was an important addition to the drugs available for treating metastatic sarcomas, when we compared the survival times of our patients who responded to adriamycin to those who did not respond, there was little clinical and no statistically significant difference (median survivals of 8 and 4 months respectively [$P = 0.43$]). Thus, efforts to further improve the therapy in this field were clearly needed.

Combination Chemotherapy With Adriamycin and NSC-45388

Having observed two new agents that were each effective in patients with advanced metastatic sarcomas, we decided to explore the possibility of using adriamycin and NSC-45388 in combination. A phase I study was begun at the M.D. Anderson Hospital to see how readily these two drugs could be combined, and at the same time basic science studies using laboratory animal tumors were begun to test the efficacy of this two-drug combination. The latter studies showed that adriamycin combined with NSC-45388 was clearly synergistic in a number of laboratory animal tumors with a minimal overlap in toxicity (7,8). The phase I study at the M.D. Anderson Hospital confirmed that the two drugs could also be used in man in nearly the full dosage with minimal toxicity complications (5).

As a result, SWOG-445 (adriamycin and NSC-45388) (table 1) was activated as a group-wide study in July 1971. During the 14-month period that this protocol was in effect, 254 sarcoma patients were entered and 218 were evaluated. The remaining 36 patients could not be evaluated largely because of major protocol violations or failure to complete one course due to death from reasons other than tumor or drug toxicity. Twenty-five complete and 67 partial remissions were observed for an overall response rate of 42%. An additional 33% of the patients were classified as having no change while 25% of the patients were not helped by the protocol and continued to have disease progression. A number of important observations were made in this study. We noted that almost all types of sarcomas responded to the regimen with the sole exception of chondrosarcoma, a tumor with an extremely poor blood supply (9) which may cause inadequate levels of drug to reach the tumor. Mesothelioma and osteogenic sarcoma were also somewhat less responsive than the other diagnoses. Because of the SWOG's prior commitment to the National Ewing's Sarcoma Study, very few patients with this diagnosis have been entered in our studies, and it is therefore difficult for us to comment about this tumor. Particularly responsive tumor types included fibrosarcoma, leiomyosarcoma, neurofibrosarcoma, and rhabdomyosarcoma (5) (table 3).

We also observed that there were some differences in the response rates according to the site of tumor origin. Thus, as shown in table 4, the response rates in head and neck, genitourinary, and uterine sarcomas were particularly high, while lesions originating in the pelvis, trunk, and gastrointestinal tract were considerably less responsive. When examined by the site of metastatic location (table 5), it was clear that nodal, subcutaneous, skin, pulmonary, and soft tissue metastases were particularly responsive, with less efficacy observed in liver, bone, and central nervous system lesions (10,11). The last-mentioned metastatic site is not frequently encountered in sarcoma. It is interesting to note, however,

TABLE 3.—Response and survival rates according to diagnosis with adriamycin alone (SWOG-442) and adriamycin plus NSC-45388 (SWOG-445)

Diagnosis	SWOG-442				SWOG-445			
	CR + PR*/No. of evaluable patients	Response rate (%)	Median survival (mos) from start of chemotherapy for— All patients	Responders only	CR + PR*/No. of evaluable patients	Response rate (%)	Median survival (mos) from start of chemotherapy for— All patients	Responders only
Angiosarcoma	0+0/2	0	2	—	1+2/9	33	14+	18+
Chondrosarcoma	0+1/3	33	3	8	0+0/12	0	12.5	—
Ewing's sarcoma	1+0/5	20	2	22+	0+1/2	50	7.5	6
Fibrosarcoma	0+2/6	33	8.5	23.5	3+9/25	48	8	13+
Leiomyosarcoma	1+1/6	33	7	7.5	7+12/38	50	13+	17+
Liposarcoma	0+0/4	0	11.5	—	3+4/18	39	9	17+
Mesothelioma	—/0	—	—	—	0+4/9	44	5	8.5
Neurofibrosarcoma	0+0/3	0	10	—	3+8/24	46	11	14
Osteogenic sarcoma	0+3/7	45	3	8	3+10/33	39	9+	11+
Rhabdomyosarcoma	0+3/9	33	5	5	3+4/18	39	8.5	13+
Synovial cell sarcoma	0+0/1	0	2	—	0+5/9	55	9	10
Undifferentiated sarcoma	0+3/3	10	12	12	2+8/21	48	7	12.5
Totals	2+13/49	31	6	8	25+67/218	42	10	16+

*CR = complete response; PR = partial response.

that in our patients who have responded or stabilized for 6 months or more, the cause of relapse has frequently been central nervous system metastasis, suggesting that patients are living longer and thus allowing this more unusual type of metastasis to appear (12).

Toxic effects associated with this regimen are the same as those expected with the single drugs used independently. NSC-45388 is responsible for a considerable amount of nausea and vomiting, especially on Day 1 when the drug is given in combination with adriamycin. Other side effects occasionally caused by NSC-45388 were a rare flu-like syndrome and probably some of the more severe cases of thrombocytopenia (see footnote 9). Adriamycin toxic effects included alopecia, rare stomatitis, and dose-limiting myelosuppression, which particularly affected the neutrophils as shown in table 6. This table also shows that the degree of myelosuppression was related to the drug dosage, with patients who tolerated the initial starting doses frequently receiving higher doses only to show a greater fall in their neutrophil count. The median day of the occurrence of the lowest white blood cell count was almost always at the end of 2 weeks, with rapid recovery thereafter, thus permitting re-treatment after a 3-week interval in all but 8% of the patients. Infectious complications were relatively rare due to the short period of myelosuppression, and no significant hemorrhagic episodes were associated with the infrequent thrombocytopenia observed (5). In all our studies with adriamycin, our total dose limit was 550 mg/m^2 and thus cardiomyopathy was extremely rare (this toxicity is discussed elsewhere [13,14]).

Perhaps our most important observation about this two-drug combination was that in addition to producing a response rate higher than that seen with the individual drugs used alone, the duration of response and particularly of survival was especially

TABLE 4.—Response according to site of primary tumor in 200 patients receiving adriamycin plus NSC-45388 (SWOG-445)

Location of primary tumor	No. of responses/No. of evaluable patients	Response rate (%)
Head and neck	11/19	58
Genitourinary and uterus	9/17	53
Retroperitoneum	13/26	50
Unknown primary and miscellaneous	17/35	49
Limb	27/70	39
Pelvis and trunk	5/19	26
Gastrointestinal tract	3/14	21

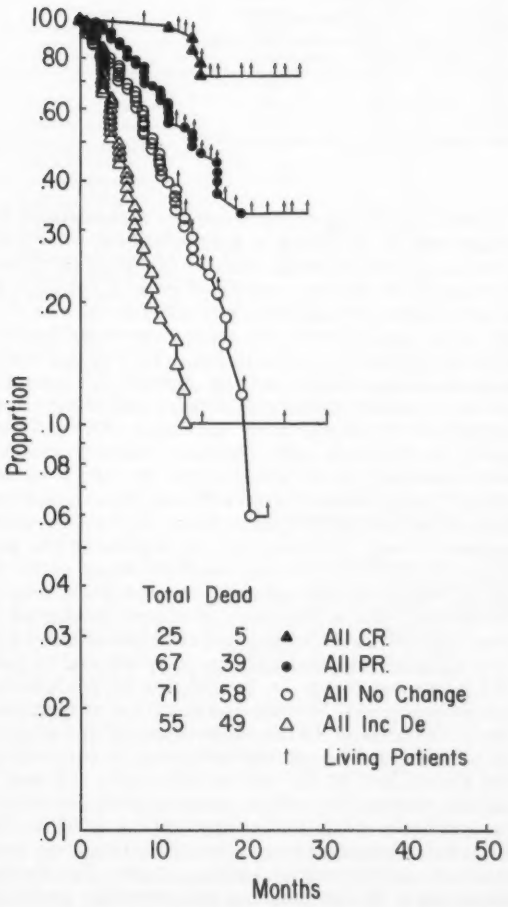

FIGURE 1. —Survival curves according to degree of response in 218 evaluable patients receiving adriamycin plus NSC-45388 (SWOG-445). Survival measured from start of treatment to death or last followup. Inc De = increasing disease patients.

TABLE 5.—Response according to site of metastases in 200 patients receiving adriamycin plus NSC-45388 (SWOG-445)

Location of metastases	No. of responses/No. of evaluable patients*	Response rate (%)
Miscellaneous	3/5	60
Nodes, subcutaneous, skin	16/29	55
Lung	60/134	45
Soft tissue	21/50	42
Liver	13/34	38
Bone	10/29	34
Central nervous system	1/7	14

*Many patients with more than one metastatic site are included at each site.

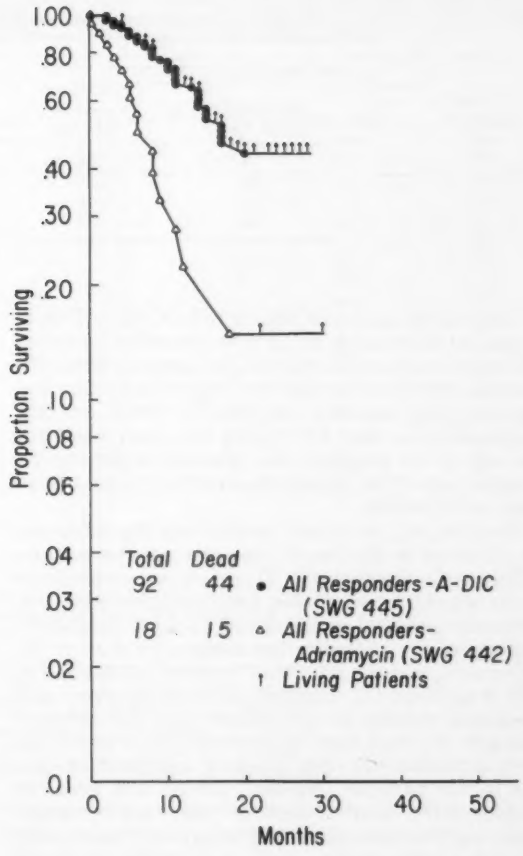

FIGURE 2.—Comparison of survival curves in patients responding to adriamycin alone (SWOG-442) (bottom line) vs patients responding to adriamycin plus NSC-45388 (A-DIC) (SWOG-445) (top line).

gratifying (fig 1). Thus, the median duration of survival from the start of chemotherapy for the complete responders will not be less than 30 months, with 20 of 25 complete responders still alive. The median duration of survival for partial responders has not yet been reached, but it cannot be less than 14 months. This is statistically inferior ($P = 0.01$) to patients achieving complete remission, but statistically superior ($P = 0.01$) to patients classified as having no change whose median duration of survival is fixed at 9 months. However, the no-change patients had a significantly ($P = 0.01$) improved survival rate over patients classified as having progressive disease whose median survival from the start of chemotherapy is fixed at 6 months, which is similar to our experience with patients failing other therapy or not receiving any therapy at all (15).

In figure 2, we show the duration of survival from the start of chemotherapy for all responders (complete or partial) receiving the two-drug combination

TABLE 6.—Leukopenia produced by adriamycin plus NSC-45388 (SWOG-445)

Dose of—		Median nadir of—		
Adriamycin (mg/m², Day 1)	NSC-45388 (mg/m²/day, Days 1-5)	Wbc (× 10³/mm³)	Neutrophils (× 10³/mm³)	Median day of nadir
45	200	3.7	2.5	15
60	250	3.4	1.9	14
75	300	3.1	0.8	13
90	350	1.5	0.4	13

of adriamycin plus NSC-45388 (SWOG-445). This is compared to the survival of all responders receiving adriamycin alone (SWOG-442). As figure 2 shows, the patients who received the two-drug combination had a statistically superior survival ($P_s = 0.001$), and this suggested to us that NSC-45388 was clearly adding, not only to our response rate, but also to our survival durations. This, therefore, seemed to be an important contribution.

However, we had to be certain that the difference we observed in the two studies was not due to some difference in the patients. Therefore, we scrupulously examined the two studies and found they were statistically identical in the distribution of diagnoses, the age of the patients, the metastatic sites of involvement, and the intervals between: (*a*) symptoms and diagnosis; (*b*) diagnosis and metastases; and most importantly (*c*) metastases and the start of therapy. We had been concerned that possibly we were administering the two-drug combination earlier in our patients' disease courses, but when we compared the interval between the onset of metastases and the start of chemotherapy for the patients receiving adriamycin alone and those receiving adriamycin plus NSC-45388, we found them to be absolutely identical. Therefore, this factor could not account for the survival differences we had observed. There were more patients who had received prior chemotherapy in the adriamycin-alone population, but the numbers of patients receiving prior chemotherapy and/or radiotherapy in the two protocols were virtually identical.

Table 3 compares, according to specific histologic diagnoses, the response rates and the median durations of survival for all patients receiving the two protocols, and also the survival time of responders receiving the two regimens. In examining this table, the superiority of the combination becomes more apparent. Thus, with adriamycin alone (SWOG-442) the median survival time of responders was equal to 1 year or more in only Ewing's sarcoma, fibrosarcoma, and undifferentiated sarcoma. With the two-drug combination (SWOG-445), median survival times in excess of 12 months were seen in angiosarcoma, fibrosarcoma, leiomyosarcoma, liposarcoma, neurofibrosarcoma, rhabdomyosarcoma, and undifferentiated sarcoma with a number of patients still alive and capable of further prolonging these medians.

It was felt that perhaps since our first adriamycin study was done during a time when we were just learning to use the drug, and the two-drug combination was done during a period of greater confidence, it would be reasonable to study adriamycin as a single drug again when we were more experienced. Therefore, a study was initiated in 1973 in which the dose-response relationship of adriamycin was explored in a wide variety of tumors including metastatic soft tissue and bony sarcomas (SWOG-7206) (table 1). Patients with adequate marrow reserve were randomly given doses of 45, 60, or 75 mg/m² every 3 weeks, and patients with inadequate marrow reserve were randomly given doses of 25 or 50 mg/m² repeated every 3 weeks (16). In examining the patients on SWOG-7206 who received doses of 50-75 mg/m² (which corresponded to the same doses used in SWOG-442, the adriamycin protocol conducted 2 years earlier), a 31% response rate was observed in 48 evaluable patients, with one complete and 14 partial responses (table 7). In addition to duplicating our response rate, the median duration of response was 3.5 months on SWOG-7206 compared to 4 months on SWOG-442. The median durations of survival of the responders in the two studies were 9.5 and 8 months respectively, which were statistically identical and again statistically inferior ($P = 0.001$) to the survival of responders receiving the two-drug combination (SWOG-445). Unlike study SWOG-442, where 63% of the patients had received previous chemotherapy, only 25% of the patients in the second adriamycin study had been given prior chemotherapy (compared to 43% in the two-drug combination). In addition, in SWOG-7206, a trend was observed which suggested that the higher the dose administered, the more likely a response would occur in sarcoma. Thus, of 33 patients receiving a dose of 75 mg/m², 12 responded for a 36% response rate compared to two of seven (29%) at a dose of 60 mg/m² and only four of 20 (20%) at a dose of 45 mg/m². This was, however, just a trend, since the chi square was not significant ($P=0.17$). Patients with inadequate marrow reserve receiving doses of 25 or 50 mg/m² had very little chance of responding to adriamycin alone, with only one response (7%) seen in 15 patients in this category.

Another way to study the effect of chemotherapy is to determine the 5-year survival from diagnosis as a result of chemotherapy. As shown in table 8, only

TABLE 7.—Response according to diagnosis in two SWOG studies using adriamycin alone

Diagnosis	SWOG-442 (phase II, 1971-72) No. of patients— Evaluable	With CR/PR*	Response rate (%)	SWOG-7206 (dose response, 1973-74) No. of patients— Evaluable	With CR/PR	Response rate (%)	Combined response rate (%)
Angiosarcoma	2	0/0	0	0	—	—	0
Chondrosarcoma	3	0/1	33	0	—	—	33
Ewing's sarcoma	5	1/0	20	2	0/0	0	14
Fibrosarcoma	6	0/2	33	7	0/2	29	31
Leiomyosarcoma	6	1/1	33	6	0/2	33	33
Liposarcoma	4	0/0	0	6	0/3	50	30
Mesothelioma	0	—	—	5	1/1	40	40
Neurofibrosarcoma	3	0/0	0	2	0/1	50	20
Osteogenic sarcoma	7	0/3	43	4	0/0	0	27
Rhabdomyosarcoma	9	0/3	33	4	0/3	75	46
Synovial cell sarcoma	1	0/0	0	2	0/0	0	0
Undifferentiated sarcoma	3	0/3	100	10	0/2	20	38
Totals	49	2/13	31	48	1/14	31	31

*CR = complete response; PR = partial response.

TABLE 8.—Comparison of 5-yr survival rates (from diagnosis) in patients receiving adriamycin alone (SWOG-442) or adriamycin plus NSC-45388 (SWOG-445)

Response to this chemotherapy	SWOG-442 Patients achieving 5-yr survival from diagnosis— At the start of this therapy (%)	As a result of this therapy (%)	SWOG-445 Patients achieving 5-yr survival from diagnosis— At the start of this therapy (%)	As a result of this therapy (%)
Responder	11	28	13	47
Nonresponder	7.5	8.5	11	16

11% of the patients who eventually responded to adriamycin alone had achieved a 5-year survival at the time adriamycin was initiated. As a result of responding to chemotherapy, a total of 28% achieved 5-year survival. On the other hand, in nonresponding patients in whom the duration of survival following chemotherapy was not significantly prolonged, there was no change in the percent of patients achieving 5-year survival. As shown in the second half of table 8, a similar proportion of patients (13%) who eventually responded to the combination of adriamycin plus NSC-45388 had already achieved 5-year survival from diagnosis at the time of initiating their chemotherapy. Because of the superior response rate and superior duration of survival produced by SWOG-445, a 47% 5-year survival was noted in the responding group as a result of the two-drug combination regimen. Again, in the nonresponding population, there was little improvement in the 5-year survival of those receiving protocol SWOG-445, since the chemotherapy added relatively little to their overall survival.

Vincristine, Adriamycin, and NSC-45388

Having observed the increase in response rates by combining the two drugs used in our earlier phase II studies, we hoped that by adding vincristine, a drug known to have some efficacy by itself in sarcomas (and which would contribute little in the way of myelosuppression), we might see further increases in our response rates. Accordingly, the SWOG activated a protocol using vincristine, adriamycin, and NSC-45388 (SWOG-7210) with the drugs administered according to the regimen described in table 1 (10,11). One hundred and twenty-four patients were registered in this study, but 17 could not be evaluated because of major protocol violations or death within the first 3 weeks before completing a single course and before remeasurement of their original tumor. Of the 107 patients that could be evaluated, nine achieved complete remission and 36 achieved partial remission, for an overall response rate of 42% (table 9). We were somewhat disappointed that we did not see an increase in the response rate by adding vin-

TABLE 9.—Response according to diagnosis in SWOG-7210 (vincristine, adriamycin, and NSC-45388) and DT 73-02 (cyclophosphamide, vincristine, adriamycin, and NSC-45388)

Diagnosis	SWOG-7210			DT 73-02		
	No. of patients—		Response rate (%)	No. of patients—		Response rate (%)
	Evaluable	With CR/PR*		Evaluable	With CR/PR	
Angiosarcoma	7	0/4	57	5	0/4	80
Chondrosarcoma	3	0/0	0	6	0/2	33
Ewing's sarcoma	5	1/1	40	1	0/0	0
Fibrosarcoma	10	1/5	60	23	4/9	57
Leiomyosarcoma	22	0/8	36	28	2/17	68
Liposarcoma	15	0/7	47	10	1/5	60
Mesothelioma	6	0/0	—	6	0/2	33
Neurofibrosarcoma	3	1/0	33	13	3/5	62
Osteogenic sarcoma	13	0/3	23	11	1/2	27
Rhabdomyosarcoma	9	2/4	67	15	6/4	67
Synovial cell sarcoma	2	0/1	50	3	0/2	67
Undifferentiated sarcoma	12	4/3	59	15	2/4	40
Totals	107	9/36	42	136	19/56	55

*CR = complete response; PR = partial response.

cristine. The patients receiving the three-drug combination seemed to be identical in all major pretreatment aspects to the patients receiving adriamycin plus NSC-45388. Although there were some shortages of adriamycin supplies in the United States at the time of this study, the response rate remained 42% even though patients who missed a course of adriamycin or received daunorubicin[10] in place of adriamycin were deleted. In some specific diagnoses, such as fibrosarcoma, rhabdomyosarcoma, and undifferentiated sarcoma (table 9), there was a suggestion that vincristine might play a small additive role, but the only significant statistical difference we observed was that the percent of patients who had progressive disease fell from 25% in the two-drug study of adriamycin plus NSC-45388 to 17% when vincristine was added ($P = 0.04$). What was more important about SWOG-7210, however, was that it confirmed the survival figures observed in our two-drug combination. Thus, the median survival was in excess of 30 months for complete responders receiving SWOG-7210 and in excess of 14 months for partial responders. These are virtually identical to the survival rates seen in the responding patients in SWOG-445. Thus, two successive SWOG studies using adriamycin plus NSC-45388 with or without vincristine had produced identical statistically superior survival patterns to two successive SWOG studies using adriamycin alone (17).

The myelosuppression observed in SWOG-7210 was identical to that seen in our earlier two-drug combination, and only 12 patients (11%) had neuropathy due to vincristine. There did not appear to be any augmented neurologic or muscular defect as might have been anticipated since vincristine is known to affect the neurologic system and adriamycin has been tentatively implicated as a toxin to the muscular system (at least with regard to cardiac muscle cells) (13, 14).

[10]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

Cyclophosphamide, Vincristine, Adriamycin, and NSC-45388

Since the addition of vincristine did not improve our response rate except possibly in the few diagnoses mentioned above, we decided to see if we could improve our overall efficacy by adding a fourth drug, cyclophosphamide, known to be active in sarcomas. Since the myelosuppression produced by cyclophosphamide is almost identical to that of adriamycin in terms of its time course, the use of these two drugs together required us to reduce the dose of adriamycin to accommodate the myelosuppression introduced by cyclophosphamide. In protocol DT 73-02 (cyclophosphamide, vincristine, adriamycin, and NSC-45388) shown in table 1, the dose of adriamycin was dropped from 60 to 50 mg/m^2 and cyclophosphamide was used at a dose of 500 mg/m^2, both given intravenously on Day 1 only and repeated every 3 weeks. The remaining parts of the protocol, ie, vincristine and NSC-45388, were left unchanged.

In the 12-month period that this protocol was active, 155 patients were accrued. Nineteen could not be evaluated for the reasons indicated in the above discussion of protocol SWOG-7210, leaving 136 evaluable patients. This study was done as an independent investigation by the Department of Developmental Therapeutics at the M.D. Anderson Hospital, but ten other institutions cooperated with us and contributed 42% of the evaluable patients (18). As shown in table 9, the overall response rate in this study was 55%, including 19 patients who achieved complete remission (14%). Since this protocol was designed mainly to determine the contribution of

cyclophosphamide, if the 12 patients in this study with prior exposure to cyclophosphamide were excluded, the complete and overall response rates would be 15% and 60% respectively, a significant improvement over our previous regimens. In examining the patient characteristics in this study (table 10), it was clear that relatively few patients had received prior chemotherapy and/or radiotherapy (55 of 136 patients or 40%), but even when we restricted the analyses of all our previous studies to patients without prior radiotherapy or chemotherapy, the superiority of our four-drug combination remained apparent. Table 10 also analyzes the responses according to the patient's sex, age, and type of prior therapy. While the patient's sex did not prognosticate for response, a finding consistent with all of our previous studies, the patients achieving complete responses did tend to have less prior therapy and to be somewhat younger. However, none of these differences were statistically significant.

With the addition of cyclophosphamide, the degree of myelosuppression observed was more significant than that seen in our previous studies, and as shown in table 11, 27% of the patients receiving DT 73-02 had an absolute granulocyte count of less than 500 cells/mm^3 sometime during the course of their chemotherapy. Because of the short duration of myelosuppression, infectious episodes were few (14%) and no deaths attributable to infection occurred. Responding patients tended to have slightly more granulocytopenia (table 11). Thrombocytopenia remained relatively insignificant, and since cyclophosphamide, like adriamycin, is known to be platelet sparing (19), the fall in platelet counts can again be attributed predominantly to NSC-45388. Only 5% of the patients had a platelet count of less than 50,000 cells/mm^3 during any part of their chemotherapy, and serious bleeding problems were not observed.

It is clear that in moving from adriamycin alone to cyclophosphamide, vincristine, adriamycin, and NSC-45388, we had improved our complete response rates from 4% to 14% and our overall response rates from 31% to 55%. Although it is too early to comment definitively on the overall survival rates of patients receiving this four-drug combination, 1 year after the close of that study only one complete and eight partial responders have died, suggesting that the overall survival rates in this study will be excellent. We have already shown that adriamycin plus NSC-45388 with or without vincristine statistically improved survival rates compared to adriamycin alone (17). Therefore, we now recommend the addition of cyclophosphamide to the three-drug regimen as the first-line approach to patients with metastatic sarcoma, until the research described below or elsewhere suggests an alteration is necessary. Until NSC-45388 is commercially available, we have recommended that practicing physicians either use the investigational program described below (cyclophosphamide, vincristine, adriamycin, and actinomycin D) in which NSC-45388 is replaced with actinomycin D, or attempt to get the NSC-45388 from the National Cancer Institute.

TABLE 10.—Patients receiving DT 73-02 (cyclophosphamide, vincristine, adriamycin, and NSC-45388)

	No. of—								
			Patients with prior therapy*			Patient age (yrs)			
Response	Evaluable patients	Men/women	Radiotherapy	Chemotherapy	Both	<20	20-40	40-60	>60
Complete	19	11/8	6	0	1	2	7	6	4
Partial	56	24/32	17	5	2	4	9	26	17
Stable	31	18/13	8	4	1	4	6	11	12
Progression	30	15/15	6	0	5	2	8	7	13
Totals	136	68/68	37	9	9	10	30	50	46

*Other than surgery.

TABLE 11.—Myelosuppression in patients receiving DT 73-02 (cyclophosphamide, vincristine, adriamycin, and NSC-45388)

	No. of patients with—					
	Absolute granulocyte nadir—			Platelet nadir—		
Response	>1000	500-1000 (cells/mm^3)	<500	>100	50-100 (cells × 10^3/mm^3)	<50
Complete	6	5	8	13	5	1
Partial	25	20	11	44	9	3
Stable	14	6	11	24	4	3
Progression	15	9	6	26	4	0
Totals	60 (44%)	40 (29%)	36 (27%)	107 (79%)	22 (16%)	7 (5%)

TABLE 12.—Regimen of ongoing protocol SWOG-7402 (cyclophosphamide, vincristine, adriamycin, and NSC-45388 versus cyclophosphamide, vincristine, adriamycin, and actinomycin D)*

Drugs	Regimen used with— Adequate marrow reserve	Inadequate marrow reserve
Regimen 1		
Cyclophosphamide	500 mg/m², Day 2 (iv)	400 mg/m², Day 2 (iv)
Vincristine	1.5 mg/m² (top dose 2 mg), Day 1 and weekly × 7 wks	1.5 mg/m² (top dose 2 mg), Day 1 and weekly × 7 wks
Adriamycin	50 mg/m², Day 2	40 mg/m², Day 2
NSC-45388	250 mg/m²/day, Days 1-5	200 mg/m²/day, Days 1-5
Regimen 2		
Cyclophosphamide	500 mg/m², Day 2 (iv)	400 mg/m², Day 2 (iv)
Vincristine	1.5 mg/m² (top dose 2 mg), Day 1 and weekly × 7 wks	1.5 mg/m² (top dose 2 mg), Day 1 and weekly × 7 wks
Adriamycin	50 mg/m², Day 2	40 mg/m², Day 2
Actinomycin D	0.3 mg/m²/day (top daily dose 0.5 mg), Days 3-5	0.2 mg/m²/day (top daily dose 0.3 mg), Days 3-5

*All patients stratified by histologic cell type and marrow reserve status prior to randomization. Regimen repeated at 3-week intervals if blood counts have recovered.

TABLE 13.—Current results of SWOG-7402 (cyclophosphamide, vincristine, adriamycin, and NSC-45388 versus cyclophosphamide, vincristine, adriamycin, and actinomycin D) in metastatic sarcomas

	Regimen 1 (cyclophosphamide, vincristine, adriamycin, and NSC-45388)	Regimen 2 (cyclophosphamide, vincristine, adriamycin, and actinomycin D)
No. of patients—		
Entered	100	96
Inevaluable	15	9
Evaluable	85	87
CR*	14	7
PR	34	37
Percent of patients entered who had—		
CR	14	7
CR + PR	48	46
Percent of evaluable patients who had—		
CR	16.5	8
CR + PR	56.5	50

*CR = complete response; PR = partial response.

Current Studies and Future Plans

When we began our investigations in metastatic sarcoma, so many of the patients referred to us had received prior chemotherapy that it was impossible to effectively investigate actinomycin D as a first-line regimen. Nevertheless, if one examines the published literature on actinomycin D used alone, it would appear to be a superior agent in sarcomas compared to NSC-45388 used alone, although the patients in which the drugs were studied were not directly comparable (20). On the other hand, animal tumor studies suggest a definite synergism with adriamycin plus NSC-45388 (7,8) while preliminary studies in advanced P388 leukemia suggest a possible antagonism between adriamycin and actinomycin D (Schabel FM, Jr., personal communication). With this conflicting data, we felt it would be reasonable to compare actinomycin D in combination with our previous regimen. Therefore, the SWOG recently activated the protocol shown in table 12 (SWOG-7402) which compares cyclophosphamide, vincristine, adriamycin, and NSC-45388 with the same combination using actinomycin D instead of NSC-45388. Several other slight changes were made in the protocol. Following the observation in L1210 leukemia that simultaneous administration of vincristine and cyclophosphamide was less effective than their sequential administration (21), cyclophosphamide and adriamycin were given the day after vincristine instead of on the same day. An attempt was also made to use vincristine in a possibly more effective schedule by giving it weekly for 7 weeks instead of on Days 1 and 5 every 3 weeks. Because actinomycin D is more myelosuppressive than NSC-45388 it can only be used at 60% of its usual full dose.

SWOG-7402 was activated in December 1973 and is currently still in progress. Table 13 shows the results of the January 1975 analysis. As indicated by the table, there is no statistical difference in the two four-drug combinations, but the arm containing NSC-45388 does have twice as high a complete response rate and a slightly superior overall response rate when compared with the arm containing actinomycin D. The median duration of complete response for patients receiving the NSC-45388 combination is 9+ months with only one patient in relapse, while for the actinomycin D study it is 6+ months, again with only one patient in relapse. The overall toxic effects

have been relatively similar to those seen in the previous NSC-45388 combination study (DT 73-02), but since actinomycin D produces stomatitis as a major side effect (which is a minor toxic effect of adriamycin), the actinomycin D combination is producing some significant mucositis not seen with the NSC-45388 combination.

Because of the encouraging results we have observed in patients with advanced metastatic disease, we felt it would be reasonable to consider using adriamycin combination chemotherapy in an adjuvant setting for patients at high risk for recurrence following extirpative but nonradical (excluding amputation) surgery and radiotherapy. The multidepartmental protocol we devised at the M.D. Anderson Hospital included the administration of adriamycin, cyclophosphamide, and vincristine given at the same time that radiotherapy was given to the surgical bed. We have limited our study to patients with soft tissue sarcomas originating in the head and neck, trunk, or extremity, with the latter lesions treated only if they are large (> 5 cm) and anaplastic with many mitoses (grades II or III). While it is too early to comment definitively on this protocol, 1 year from its initiation only one patient has relapsed. Tolerance to this combined modality approach has been generally excellent, but skin breakdown problems have occurred and we are still adjusting our schedules.

In our most recent investigations we are exploring the use of a protected environment for patients with metastatic sarcoma in the hope that protective isolation will permit the use of larger doses of myelosuppressive chemotherapy which will in turn produce better responses. Of the eight patients treated thus far, all have done well without tumor progression, but further comment is not possible at this time.

Table 14 is a summary of all the studies containing adriamycin discussed in this article. Apart from Ewing's sarcoma where the SWOG's previous commitment to the National Ewing's Sarcoma Study has already been mentioned, there are 25 or more patients in each of the major sarcoma diagnoses, and response rates near 50% have been reached in all but four of these diagnoses. Chondrosarcoma remains an extremely refractory tumor, although some responses are now being seen with the four-drug combinations. Our poor results in Ewing's sarcoma may reflect predominately the advanced stage of the patients we receive in our studies. Mesothelioma, which resembles sarcoma histologically in some of its presentations, is another difficult tumor, although some progress appears to be occurring with the combinations, particularly with respect to response rates and survival durations. With osteogenic sarcoma it is difficult to be certain that the combinations have added much, if anything, to the response rate seen with adriamycin alone, but we have observed superior survival with the combinations. We have also confirmed the findings of Cortes et al (22), that osteogenic sarcoma patients who have a long interval between their diagnosis and the appearance of metastases are much more likely to respond than patients with short intervals. In addition, we have observed that osteoblastic osteogenic sarcoma seems to be considerably more responsive than chondroblastic osteogenic sarcoma, with the latter apparently sharing the refractoriness of chondrosarcoma. In DT 73-02 the number of patients with the chondroblastic subtype of osteogenic sarcoma was particularly high, which may account for the poor response rate for osteogenic sarcoma patients in that study. The undifferentiated group of sarcomas includes a large number of mixed mesodermal tumors, which, when they arise in the uterus, are

TABLE 14.—Results of SWOG and M.D. Anderson sarcoma studies with adriamycin alone and in combination (1971-74)

Diagnosis	No. of patients—* Evaluable	With CR	With PR	Response rate† (%)
Angiosarcoma	35	3	17	57
Chondrosarcoma	29	1	4	17
Ewing's sarcoma	15	2	2	27
Fibrosarcoma	90	9	37	51
Leiomyosarcoma	147	14	60	50
Liposarcoma	71	7	28	49
Mesothelioma	36	3	9	33
Neurofibrosarcoma	50	7	15	44
Osteogenic sarcoma	87	5	23	32
Rhabdomyosarcoma	69	13	25	55
Synovial cell sarcoma	25	4	10	56
Undifferentiated sarcoma	76	9	27	47
	730	77	257	46

*CR = complete response; PR = partial response.
†Overall CR rate = 11%.

quite responsive to these combination regimens as shown in table 4. However, there are a group of patients included under undifferentiated sarcomas that are only described by pathologists with the statement that an undifferentiated sarcoma cannot be excluded. Undoubtedly, we are including in this large category some patients who probably don't have sarcomas, which may reflect the somewhat lower response rate of this group of tumors in our more recent studies.

CONCLUSIONS

While it is clear that considerably more progress is needed, in the 5 years that adriamycin has been in clinical trials significant advances have been made in the development of chemotherapeutic regimens available for adult patients with many different types of metastatic sarcomas. Combination chemotherapy with adriamycin has significantly improved not only response rates but also response and survival durations. It can be hoped that in some of the adjuvant studies discussed in the last part of this paper, as well as in other papers in this symposium covering the adjuvant treatment of osteogenic sarcoma, goals that reach beyond extended palliation can be achieved.

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Adriamycin (NSC-123127) Therapy in Thyroid Carcinoma[1,2]

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SUMMARY

Forty-three patients with progressive, metastatic, or refractory thyroid carcinoma received adriamycin at doses ranging from 45 to 90 mg/m² repeated every 3 weeks. Of the 43 patients, 15 (35%) had a greater than 50% reduction in their tumor masses which was associated with a significantly increased survival. The median duration of response is 7+ months (range, 2-39 months) with five patients still responding. The median survival times from the start of chemotherapy are 20+ months (range, 5-43+ months) for the 15 responding patients and 5 months (range, 3 days to 38 months) for the 28 nonresponding patients ($P = < 0.01$). Pulmonary metastases were the most responsive followed by bone and soft tissue of the neck lesions. While all histologic types of thyroid carcinoma responded, medullary and Hürthle cell carcinoma were the most responsive, and adriamycin may be particularly useful in the former diagnosis which has previously been notoriously refractory to all types of therapy. Spindle and giant cell carcinoma, particularly when it involved the neck, was the least responsive to adriamycin, and efforts to improve the response rate for this group of patients are currently in progress.

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Thyroid cancer is a relatively rare tumor affecting predominantly an elderly population. Usually surgery, consisting of subtotal or total thyroidectomy, is adequate treatment, and long-term survival consistent with cure is recorded in many series following such procedures. In some patients the extent of local tumor or recurrent disease requires the addition of radiotherapy which may consist of internal radiation (ie, the use of ^{131}I) or external beam conventional radiotherapy. The former is usually reserved for patients whose tumors demonstrate concentration of the isotope, which may occur not only in lesions recurring in the neck but in distant metastases as well. In other patients, significant palliation is readily achieved with a circumscribed port of external beam radiotherapy (1,2).

Nevertheless, a group of patients exist for whom these therapeutic modalities are inadequate. These are patients who *(a)* have diseases which are usually unresponsive to surgery or radiotherapy, such as spindle and giant cell carcinoma or medullary carcinoma; *(b)* have recurrent disease in the neck despite extensive surgery and radiotherapy; or *(c)* have distant diffuse advancing metastases no longer responsive to the above radiotherapeutic or surgical approaches. Prior to the introduction of adriamycin, patients in the above categories referred to medical oncologists for systemic chemotherapy did very poorly. We were unable to find any consistently active drug capable of producing significant tumor regression associated with a prolongation in survival in a review conducted in 1970 of our own experience at the M. D. Anderson Hospital along with a review of the recorded literature (3). However, during the course of that review, we noted that three of the first five patients treated with adriamycin (a drug then undergoing a phase I trial at our institution) showed some response. We decided to carry out a further prospective trial in which patients with progressive thyroid carcinoma refractory to other therapeutic measures would receive adriamycin as their first chemotherapeutic agent.

This paper is a summary of the results of these studies and includes the first five patients in our previously mentioned review paper (3), as well as an

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[2]Adriamycin was supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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additional 25 patients described in an interim report recently published (4). The present analysis consists of 43 patients, 30 of whom have been previously described. It should also be noted that eight of these patients are included in the ten nonresponders described in the Southwest Oncology Group (SWOG) phase II study report on adriamycin (5), and several others are currently in the SWOG adriamycin dose-response study (6). The final results regarding specific diagnoses in this latter study have not yet been reported.

METHODS AND MATERIALS

Patient Population

Forty-three patients with progressive refractory thyroid cancer metastatic to the local soft or nodal tissue of the neck or to distant sites were treated with adriamycin during the 4-year period from January 1971 to December 1974 (see appendix). There were 20 men and 23 women ranging in age from 40 to 80 years with a median age of 64 years. One man was black and the remainder of the patients were white.

Tumor types were classified according to previously published criteria (7) as follows: *(a)* pure papillary (two patients); *(b)* pure follicular (four patients); *(c)* mixed follicular-papillary (seven patients); *(d)* medullary (solid) (six patients); *(e)* Hürthle cell (nine patients); and *(f)* spindle and giant cell, either pure or mixed with papillary or follicular carcinoma (14 patients). One patient's tumor could not be histologically classified. In five patients, spindle and giant cell carcinoma had developed at the time adriamycin therapy was started although mixed papillary and follicular carcinoma had been diagnosed earlier. The proportion of patients with either the medullary or the spindle and giant cell varieties (20 of 43 patients, 47%) was considerably higher in this population than in our population of thyroid cancer as a whole, indicating the more aggressive nature of these tumors and their more frequent need for chemotherapy.

Of the 43 patients, 41 had been treated surgically either by a subtotal thyroidectomy (13 patients, 30%) or a total thyroidectomy (28 patients, 65%). In addition, 16 patients (37%) had undergone radical neck dissection. Two patients with very rapidly progressing spindle and giant cell carcinoma, who already had pulmonary metastases at initial presentation, had a diagnostic needle biopsy only. A radiotherapeutic approach had been used in 32 patients consisting of external beam megavoltage therapy in 15 patients (35%), ^{131}I administration in five patients (12%), or both in 12 patients (28%). Seven patients (16%) had received prior chemotherapeutic agents. Metastases beyond the soft tissue of the neck were present in all but four patients: 34 patients with pulmonary (79%), 14 patients with bony (39%), and three patients with hepatic (7%) involvement (see appendix).

Treatment Regimen

Adriamycin was supplied in ampules containing 10 mg. The drug was reconstituted with 20-50 ml of normal saline or 5% dextrose and water, and was given as a direct intravenous (iv) injection or as a rapid iv infusion over 10-15 minutes with care taken to avoid extravasation to prevent the drug's vesicant effect. The usual starting dose was 75 mg/m^2 as a single injection (or occasionally divided into three consecutive daily injections early in the course) repeated at 3-week intervals. For patients with extensive prior therapy, a starting dose of 60 mg/m^2 was used. Three patients with rapidly advancing spindle and giant cell carcinoma were treated with initial doses of 90 mg/m^2. Four patients received starting doses of 45 mg/m^2 as part of a study performed in cooperation with the SWOG investigating the dose-response relationship of adriamycin (in which patients were assigned to receive doses of either 45 or 75 mg/m^2 every 3 weeks) (6). Except for these four patients whose dosage was fixed, the doses of subsequent courses in other patients were raised or lowered by an increment of 15 mg/m^2 according to the degree of myelosuppression seen in the previous 3 weeks. These adjustments were done with a goal of keeping the maximum myelosuppression in the range of 1500 absolute leukocytes/mm^3 and/or 75,000 platelets/mm^3. A course was defined as a single injection followed by a 3-week observation period. Therapy was continued every 3 weeks until clear-cut progression was observed or toxic effects precluded further drug administration. One year after therapy started, the increased incidence of adriamycin-induced cardiomyopathy associated with high cumulative doses of adriamycin was recognized (8,9), and the total dose of adriamycin delivered was subsequently limited to 550 mg/m^2.

To be eligible for the study, a starting white blood cell count of greater than 4000 cells/mm^3 and a platelet count of greater than 100,000 cells/mm^3 were required, along with a measurable lesion and a history of no prior exposure to adriamycin or daunorubicin. All patients signed a consent form indicating an understanding of the investigational nature of adriamycin therapy. Chemotherapy was administered on an outpatient basis unless precluded by the patient's physical condition. Toxic and therapeutic effects of the regimen were monitored by weekly blood counts including differential and platelet counts. In addition, tumor measurements, renal and hepatic function tests, chest roentgenograms, electrocardiograms, and enzyme determinations (creatinine phosphokinase, serum glutamic oxaloacetic transaminase, and lactic dehydrogenase) were performed at 3-week intervals. Appropriate radiologic studies to follow tumor responses were repeated at least every 2 months.

Patients showing an objective response lasting for 1 month or more were classified as having a partial remission if they had a 50% or greater decrease

in the sum of the products of the largest perpendicular diameters of all measurable lesions without a simultaneous increase in the size of any lesion or the occurrence of any new metastases. Statistical analysis was performed with a generalized Wilcoxon-Gehan test (10) and a P value of less than 0.05 was considered significant. Followup observations for all 43 patients have been completed, with analyses of the 13 living patients terminated as of December 31, 1974.

RESULTS

Of the 43 patients, 15 (35%) achieved a partial remission, with a median of three courses required to produce this degree of regression. All responses occurred by seven courses, but no response was seen before completion of two courses. Seven patients died before receiving or completing their second course of adriamycin. Regressions were occasionally dramatic, not only objectively (figs 1 and 2) but also subjectively, with relief of bone pain being especially impressive. In four of the 13 responders the response was nearly complete with minimal residual disease documented only by repeated biopsy. One patient who was confined to bed at the start of therapy because of pain from extensive metastatic involvement of the lower proximal limbs and the pelvis was able to resume full ambulation with a near-normal gait without assistance. Remissions occurred most frequently in medullary and Hürthle cell carcinomas, but were longest in medullary and papillary-follicular carcinomas (table 1). The most responsive lesions were pulmonary metastases followed by metastases to the bone and the nodal or soft tissue of the neck in that order. None of the three patients with hepatic involvement showed regression in these metastases (table 2). While the median duration of response was only 7+ months (range, 2-39 months), five of the 15 responding patients are still in remission, and several can still affect the median. Survival of the 15 responders was significantly superior to that of the 28 nonresponding patients (fig 3) with 20+ and 5 months' median survivals respectively. Table 3 shows that responders lived longer than patients whose disease was considered stable or who had had less than 50% regression (classified as "no change" patients). The latter patients lived longer than the patients who had disease progression, with these patients subdivided into two groups—those who completed two or more courses (called "progressors" or "progression" patients) and those who probably did not have a chance to respond because they died before receiving or completing a second course (called "early deaths"). This trend held for all cell types with the sole exception of the two patients with progressive Hürthle cell carcinoma, who had a median survival 1½ months longer than the four patients with stable disease in that same histologic category. When responders are compared to nonresponders as a whole, the difference in survival favors responders significantly ($P<0.01$), but there is no statistical difference ($P = 0.23$) when responders are compared only to patients classified as having no change. Figure 4 graphically shows that both responding patients and no change patients have survival rates significantly superior to those of patients with progressive disease or early deaths while receiving adriamycin therapy. Only four of the 18 patients (22%) with prior ^{131}I treatment responded. With this exception there was no other significant difference in the responding and nonresponding groups including age or extent of the disease at the start of adriamycin therapy. Our previous paper (4) compared the rate of disease progression in responding and nonresponding patients. Although intervals between symptoms and diagnosis and between diagnosis and metastases were slightly longer in responding patients, a statistical evaluation of these intervals showed that the differences were not significant, and the median interval between diagnosis and the start of adriamycin therapy in the two groups was virtually identical at 26 months.

Eight of the 12 patients who showed neither progression nor the required amount of regression in their disease to be called partial remissions probably benefited clinically from their adriamycin therapy, because the rapidly progressive disease course which had been seen before the start of adriamycin therapy was clearly halted. While only two of the six patients with medullary carcinoma had associated diarrhea, in both patients the diarrhea cleared completely, but no significant changes were observed in their measurable disease. All 43 patients were euthyroid at the time adriamycin therapy was started because of exogenous replacement, and they remained so during the study.

TABLE 1.—Duration of adriamycin-induced responses in thyroid carcinoma patients

Cell type	No. of responders/ No. of patients treated	Response (%)	No. of patients still in remission	Duration of response (mos)	
				Median	Range
Spindle and giant cell	4/14	29	1	6.5+	3-28
Papillary-follicular	4/13	31	2	13.5+	4-21+
Hürthle cell	3/9	33	1	9	7-16+
Medullary (solid)	3/6	50	1	21+	2-39
Unclassified	1/1	100	0	6	—
Totals	15/43	35	5	7+	2-39

TABLE 2.—Response according to metastatic site

Type of metastases	No. of patients*	No. of responses	Response rate (%)
Pulmonary	34	12	35
Soft tissue (neck)	22	5	23
Bone	14	4	29
Hepatic	3	0	0

*Several patients are listed more than once because they had metastatic involvement of multiple sites.

TABLE 3.—Thyroid carcinoma: response to adriamycin and subsequent survival according to histologic cell type

	Cell type*					
Response category	Spindle and giant cell	Papillary-follicular	Hürthle cell	Medullary (solid)	Unclassified	Totals
Response (CR or PR)						
No. of patients	4	4	3	3	1	15
Median survival in mos (range)	9+(5–43+)	19+(12+–26+)	19+(10–43)	26+(11–43)	10	20+(5–43+)
No-change						
No. of patients	1	5	4	2	0	12
Median survival in mos (range)	6	13+(8+–38)	8(5–34+)	15.5+(13+–18)	—	14+(5–38)
Progression						
No. of patients	4	3	2	0	0	9
Median survival in mos (range)	3.5(1–5)	5(5–7)	9.5+(9+–10+)	—	—	4(1–10+)
Early death						
No. of patients	5	1	0	1	0	7
Median survival in mos (range)	0(11 days–1 mo)	1	—	3 days	—	0(3 days–1 mo)
Total						
No. of patients	14	13	9	6	1	43
Median survival in mos (range)	4.5(0–43+)	12+(1–38)	10+(5–34+)	15.5+(0–43)	10	10(0–43+)

*_Spindle and giant cell_: 2 responders still alive.
Papillary-follicular: 2 responders and 2 no-change patients still alive.
Hürthle cell: 2 responders, 1 no-change patient, and 2 progressors still alive.
Medullary (solid): 1 responder and 1 no-change patient still alive.
All types: 7 responders, 4 no-change patients, and 2 progressors still alive.

Of the 16 patients starting at doses of 60 mg/m^2 of adriamycin every 3 weeks, eight had their doses increased to 75 mg/m^2 or more. In this group, there were six responses: three at doses of 60 mg/m^2 alone and three in the dose-escalation group. Five of the 20 patients initially treated at doses of 75 mg/m^2 had their doses increased. Responses were seen in six patients at a dose of 75 mg/m^2 alone and in three of five patients in the dose-escalation group. None of the patients initially receiving doses of 45 mg/m^2 responded (no dose changes were permitted by protocol), and three moribund patients with spindle and giant cell carcinoma died of their disease 11, 18, and 36 days respectively after receiving starting doses of 90 mg/m^2.

Toxic Effects

Adriamycin therapy was for the most part well tolerated. Granulocytopenia in 36 patients (84%) was the most consistently seen side effect but dose escalation was built into the protocol in an attempt to ensure evidence of biologic activity. It is important to note that none of the seven patients in whom leukopenia failed to develop showed any tumor response. Maximum granulocytopenia occurred approximately 2 weeks after each course of adriamycin, with prompt recovery permitting re-treatment at 3-week intervals in almost all patients. No definite infectious episodes associated with leukopenia were identified. Although alopecia was almost total in all patients surviving 6 weeks or more, it was always reversible and it did not represent a serious psychologic problem. Nausea and vomiting, usually limited to the day of drug administration, occurred in 23 patients (53%), with anorexia occasionally persisting for several days. Fever on the day of administration, phlebitis at the injection site, and moderate buccal stomatitis were rarely observed and were never dose limiting. Thrombocytopenia occurred in only seven patients (16%), and the lowest platelet

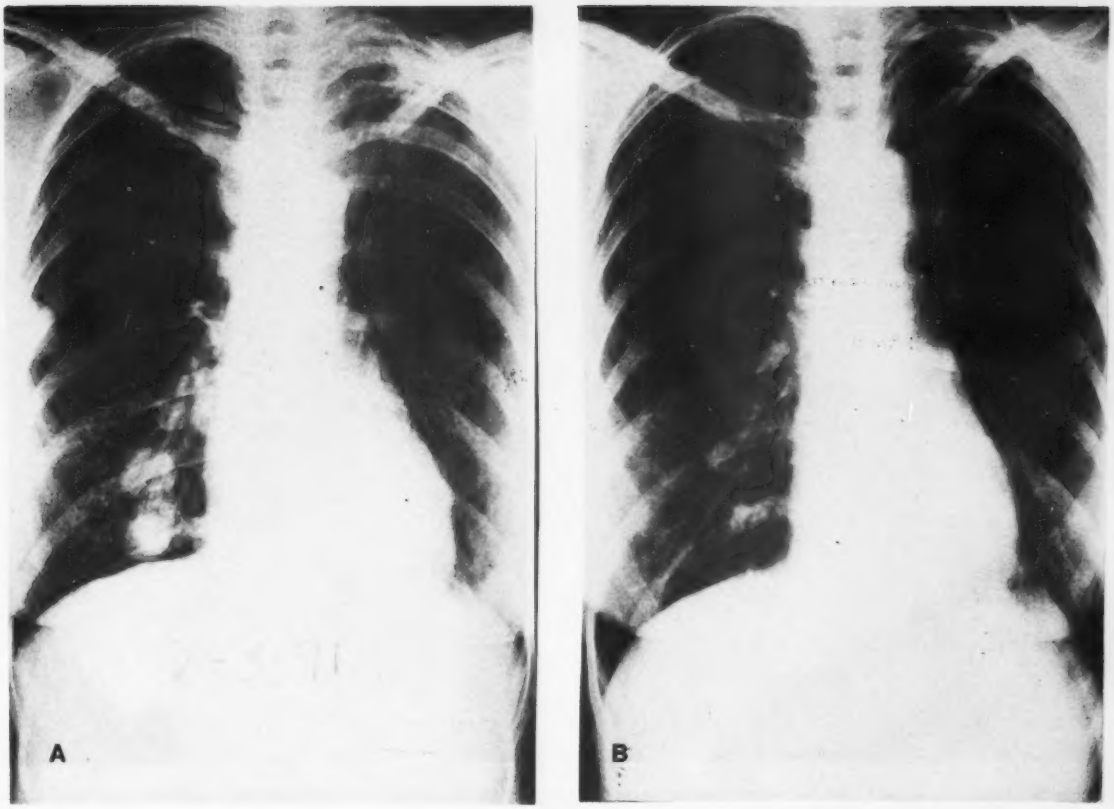

FIGURE 1.—(A) Posteroanterior chest roentgenogram before adriamycin therapy in a 54-year-old woman with Hürthle cell thyroid carcinoma. (B) Posteroanterior chest roentgenogram 8 months after adriamycin therapy.

count recorded in the entire series was 34,000 cells/mm^3. No platelet transfusions were required.

The most formidable toxic effect encountered was the adriamycin-induced cardiomyopathy. During the first part of this study, three patients received total doses of adriamycin in excess of 550 mg/m^2. In two of them, severe refractory congestive heart failure developed, from which they clinically recovered, but they were never able to resume full physical activity. A third patient, who received a dose of 895 mg/m^2, died suddenly while granulocytopenic, and although an autopsy was not performed and sepsis cannot be ruled out, it was our opinion that her death was probably secondary to adriamycin-induced cardiomyopathy. Since we recognized the need to limit patients to a total adriamycin dose of 550 mg/m^2 (8,9), cardiomyopathy has not developed in any of the 16 patients subjected to this dose limitation.

DISCUSSION

Between March 1944 and December 1974, 1039 patients with thyroid cancer have been seen at the M.D. Anderson Hospital. In 240 patients (23%) recurrent or metastatic disease developed, but only 84 patients were treated with chemotherapy. While the number of patients receiving chemotherapy for refractory or metastatic thyroid carcinoma has been small, the major reason that few patients received chemotherapy in the past has been the unavailability of an effective regimen to offer them (3). The present investigation suggests that adriamycin therapy may now provide such a regimen. Of the 43 patients treated, 15 (35%) achieved a partial remission, which was usually associated with gratifying subjective improvement. It should also be emphasized that seven patients died before the completion of at least two courses; these patients probably did not have a chance to respond to drug therapy since this was the minimal time period required for any other patient to show response. They have been considered early deaths in figure 4, and their rapid rate of death is clearly indicated by the steep slope in that graph. The response rate of the 36 patients receiving two or more courses was 42%. Survival of

FIGURE 2.—(A) Soft tissue neck mass before adriamycin therapy in same patient described in fig 1. (B) Soft tissue neck mass 6 months after adriamycin therapy.

patients after the start of adriamycin therapy was related to response, with the responders having a significantly longer survival time than the nonresponders considered as a whole or with early-death patients excluded. However, patients who achieved stabilization of previously progressive disease also showed improvement in their survival rates which were statistically similar to those of responding patients and definitely superior to the survival rates of patients with progressive disease. This survival pattern was observed in all histologic types of thyroid cancer, except Hürthle cell carcinoma. While all cell types responded, the spindle and giant cell variety, the most aggressive form of this disease, appeared to be less responsive than the other varieties of thyroid cancer. This was particularly true with patients who had disease involving the soft tissue of the neck. Perhaps our most important observations were made in patients with medullary carcinoma, a tumor notorious for its refractoriness to therapy (11). Of the six patients treated, one died 3 days after starting therapy and could not be evaluated. Of the remaining five patients, there were three partial remissions. Disease-related diarrhea disappeared in the other two patients, suggesting that adriamycin therapy may be especially worthwhile against this particular histologic subtype.

The responses of nine of 23 patients (39%) receiving doses of 75 mg/m^2 of adriamycin every 3 weeks, and of three of eight patients (38%) with inadequate marrow reserve receiving doses of 60 mg/m^2 of adriamycin appeared to be identical. Of the five patients receiving doses subsequently escalated from 75 to 90 mg/m^2, three (60%) responded. It may be particularly worthwhile to explore this dose in diseases that are progressing rapidly at the time therapy starts.

After the completion of adriamycin therapy (550 mg/m^2), six patients with good partial responses were unmaintained until evidence of disease progression was shown, at which time they received an investigational drug, usually methyl-CCNU. In these six patients the median duration from the completion of adriamycin therapy until the disease progression required the start of another drug was 9+ months (range, 4-14 months). Patients failing adriamycin therapy initially or still having a large amount of tumor at the completion of adriamycin therapy were immediately switched to new therapy.

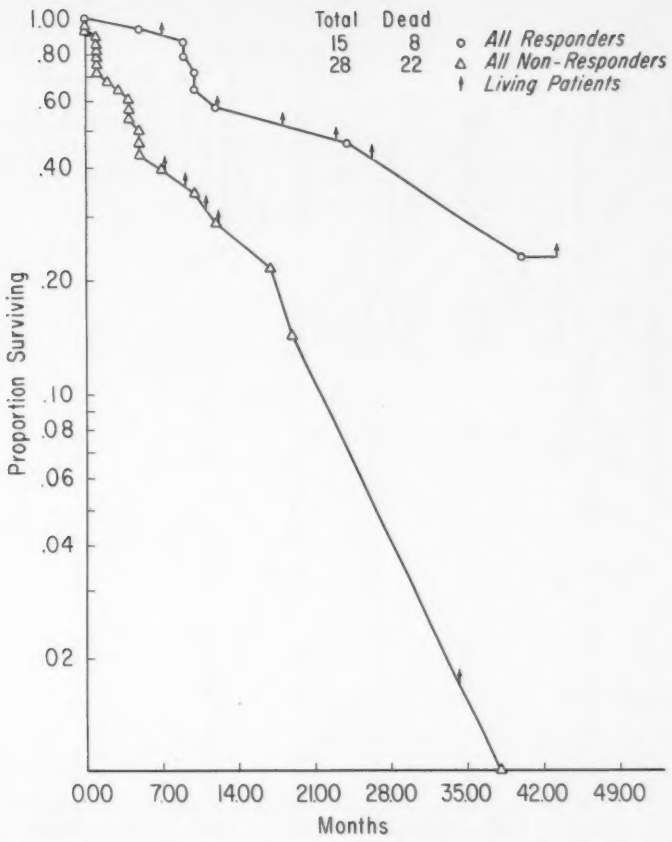

FIGURE 3.—Survival curves of responders and nonresponders with thyroid carcinoma from the start of adriamycin therapy.

We are frequently asked what to do for thyroid cancer patients who have failed or have utilized their full dose of adriamycin. Of the 12 patients who received methyl-CCNU (200 or 150 mg/m^2 orally every 6 weeks for adequate and inadequate marrow reserve patients respectively), six had stabilization of previously progressive disease (three of four with papillary-follicular, two of three with medullary, and one of one with Hürthle cell), including two patients who remain on therapy. Three additional patients with spindle and giant cell carcinoma and one unclassified patient failed to respond to methyl-CCNU. Our remaining subsequent investigational therapy is summarized in table 4, but does not yet reveal a significant trend indicating which agent might be useful in this setting. Higby et al (12) recently reported a response in an anaplastic thyroid carcinoma patient who received *cis*-dichlorodiammineplatinum(II). Short-lasting responses have also been seen by other investigators using bleomycin (13), and these two agents may deserve further separate trials. Laboratory studies indicating synergism in animal tumors (14) with cyclophosphamide, which has occasionally produced responses in thyroid cancer in man (15), and *cis*-dichlorodiammineplatinum(II) suggest that this combination might also be worth investigating as a backup regimen when adriamycin therapy has failed.

We are most distressed with our poor response rate with spindle and giant cell carcinoma invading the soft tissue of the neck where nine of 11 patients progressed. Because of the high growth fraction of this tumor, we have added methotrexate, a cell-cycle-specific agent, at a dose of 30 mg/m^2 iv weekly to adriamycin at a dose of 90 mg/m^2 given every 3 weeks for this disease. It is too early to comment on the efficacy of this combination yet, but stomatitis is clearly a problem, and patients may require nasogastric or iv hyperalimentation (which is often obligatory due to the local obstruction produced by the tumor in the neck).

Two other institutions, the National Cancer Institute in Milan and the Roswell Park Memorial Institute, have reported in this symposium similar results using adriamycin in a smaller series of patients with this disease, and although preliminary, the results of their studies and ours are encouraging and suggest that adriamycin should be explored in additional patients with metastatic thyroid carcinoma.

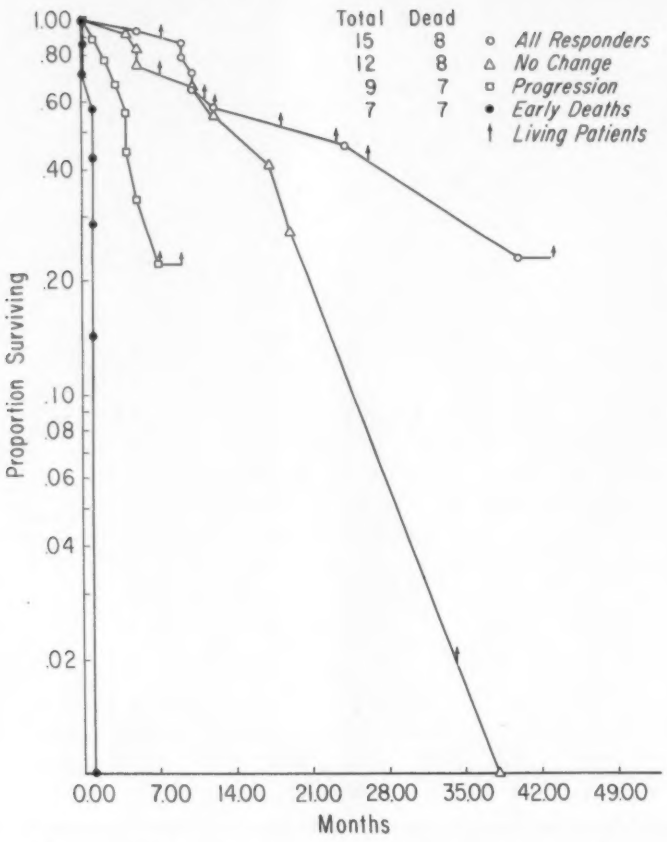

FIGURE 4.—Survival curves according to degree of response to adriamycin.

TABLE 4.—Later therapy (other than methyl-CCNU) received by patients subsequently or initially refractory to adriamycin

Agents	NSC No.	Results
Chemotherapy		
Asaley	167780	1 HC patient stabilized and 1 M patient progressed
Piperazinedione	135758	1 PF and 1 M patient progressed
Baker's antifol	139105	1 HC patient stabilized
Cyclophosphamide	26271	1 PF patient stabilized
Bleomycin	125066	1 M patient progressed
Immunotherapy		
MER	143769	1 SG and 1 HC patient stabilized
Corynebacterium parvum (IV)		1 M patient progressed

*HC = Hürthle cell carcinoma; M = medullary (solid) carcinoma; PF = papillary-follicular carcinoma; SG = spindle and giant cell carcinoma.

APPENDIX.—Patient population

Patient No.	Age/ Sex	Prior therapy	Metastatic involvement	Onset of symptoms	Date of diagnosis	Metastases developed	Adriamycin started	Date of death	Response	Comments
Mixed papillary and follicular										
4	69/M	Total thyroidectomy; ^{131}I;radiotherapy	Pulmonary	Mar 1966	June 1966	Jan 1970	Sept 1970	Sept 1971	Partial for 4 mos	—
10	69/M	Subtotal thyroidectomy;radical neck dissection;^{131}I; radiotherapy	Soft tissue; pulmonary	Mar 1965	Mar 1965	Dec 1969	Apr 1971	Aug 1971	Increasing disease	—
20	66/F	Subtotal thyroidectomy; ^{131}I;radiotherapy	Bone;pulmonary	Apr 1952	June 1952	June 1962	Mar 1972	Mar 1973	No change	Stabilization of previously increasing disease
22	60/F	Total thyroidectomy	Pulmonary	June 1966	Apr 1970	Oct 1972	Nov 1972	Living	Partial for 21+ mos	Asymptomatic
23	60/M	Subtotal thyroidectomy;^{131}I; radiotherapy	Soft tissue; bone;pulmonary	June 1959	June 1961	July 1969	Nov 1972	May 1973	Increasing disease	Died of cerebral metastases
33	44/F	Subtotal thyroidectomy;^{131}I; radiotherapy	Pulmonary	Dec 1962	Feb 1963	Dec 1973	Jan 1974	Living	Partial for 9+ mos	Now receiving methyl-CCNU
36	57/M	Subtotal thyroidectomy;radical neck dissection	Soft tissue; pulmonary	Dec 1973	Jan 1974	Feb 1974	May 1974	Living	No change	Now receiving methyl-CCNU
Follicular										
8	57/F	Total thyroidectomy; ^{131}I;radiotherapy	Soft tissue; bone; pulmonary	Jan 1969	Apr 1969	July 1969	Mar 1971	July 1971	Increasing disease	—
9	52/F	Total thyroidectomy; ^{131}I;radiotherapy	Bone	Aug 1967	Jan 1968	Aug 1968	Apr 1971	Apr 1973	Partial for 18 mos	Recovered from adriamycin-induced cardiomyopathy
15	52/F	Total thyroidectomy	Pulmonary	Jan 1969	Nov 1970	Nov 1970	Sept 1971	Nov 1974	No change	Stabilization of previously progressing disease;

APPENDIX.—Patient population—Continued

Patient No.	Age/ sex	Prior therapy	Metastatic involvement	Onset of symptoms	Date of diagnosis	Metastases developed	Adriamycin started	Date of death	Response	Comments
										remained stable on methyl-CCNU; progressed on piperazinedione
38	75/F	Subtotal thyroidectomy;^{131}I	Pulmonary	Sept 1966	Nov 1966	Nov 1973	Jan 1974	Living	No change	Now receiving cyclophosphamide
Papillary										
16	60/M	Total thyroidectomy; radical neck dissection; ^{131}I	Pulmonary	May 1962	May 1962	Apr 1964	Dec 1971	Apr 1973	No change	Stabilization of previously progressing disease failed on subsequent methyl-CCNU;recovered from adriamycin-induced cardiomyopathy
18	75/M	Total thyroidectomy; radical neck dissection; radiotherapy	Bone;pulmonary	June 1957	June 1957	May 1969	Sept 1971	Oct 1971	Increasing disease	Died 33 days after start of adriamycin
Giant and spindle cell										
3	63/M	Total thyroidectomy	Soft tissue; pulmonary	Mar 1970	June 1970	Aug 1970	Aug 1970	Sept 1970	Increasing disease	Died 17 days after start of adriamycin
11	67/F	Total thyroidectomy; radical neck dissection	Soft tissue; pulmonary	Feb 1971	Mar 1971	Mar 1971	May 1971	May 1971	Increasing disease	Died 18 days after start of adriamycin
12	75/F	Total thyroidectomy	Soft tissue	Feb 1971	Mar 1971	Apr 1971	May 1971	July 1971	Increasing disease	Initial transient response
13	71/F	Subtotal thyroidectomy;radical neck dissection;^{131}I;methotrexate	Pulmonary	Mar 1970	Apr 1970	May 1971	June 1971	Living	Partial for 28 mos	Prior transient response to methotrexate
14	72/F	Total thyroidectomy; ^{131}I	Soft tissue; pulmonary	Oct 1970	Jan 1971	Feb 1971	July 1971	Oct 1971	Increasing disease	—

17	70/F	Total thyroidectomy; radical neck dissection; ^{131}I;radiotherapy;5-FU	Soft tissue	May 1966	May 1966	May 1966	Jan 1971	Feb 1971	Increasing disease	Died 30 days after start of adriamycin
21	64/M	Total thyroidectomy; radical neck dissection	Soft tissue; pulmonary	Jan 1971	Apr 1972	July 1972	July 1972	Dec 1972	No change	Failed on subsequent methyl-CCNU
25	67/F	Subtotal thyroidectomy; radiotherapy	Pulmonary	Apr 1967	Apr 1972	Apr 1972	Feb 1973	Oct 1973	Partial for 7 mos	—
26	58/M	Total thyroidectomy; ^{131}I;radiotherapy	Pulmonary	Oct 1972	Oct 1972	Feb 1973	Mar 1973	July 1973	Increasing disease	Failed on subsequent methyl-CCNU
32	66/F	Needle biopsy only	Soft tissue; pulmonary	July 1973	Oct 1973	Oct 1973	Oct 1973	Nov 1973	Increasing disease	Died 36 days after start of adriamycin
34	71/M	Total thyroidectomy; radiotherapy	Soft tissue; pulmonary	Dec 1969	June 1973	July 1973	Dec 1973	Apr 1974	Partial for 3 mos	Failed on subsequent methyl-CCNU
35	70/F	Subtotal thyroidectomy;radiotherapy	Soft tissue	Jan 1974	Mar 1974	Mar 1974	Apr 1974	Living	Near complete remission for 6+ mos	On immunotherapy now
41	80/F	Needle biopsy only	Soft tissue; pulmonary	July 1974	Oct 1974	Oct 1974	Oct 1974	Oct 1974	Increasing disease	Died 11 days after start of adriamycin
42	76/F	Total thyroidectomy; ^{131}I;radiotherapy	Soft tissue; pulmonary	Feb 1968	Apr 1968	Aug 1968	Oct 1974	Nov 1974	Increasing disease	Methotrexate added, but died 20 days after start of adriamycin
Medullary (solid)										
1	51/M	Subtotal thyroidectomy; radiotherapy; multiple drug therapy	Soft tissue; pulmonary	Feb 1964	Feb 1964	Mar 1964	June 1970	Apr 1971	Partial for 2 mos	Failed on subsequent bleomycin
2	56/M	Total thyroidectomy; radiotherapy	Bone;pulmonary	June 1969	Aug 1969	Mar 1970	Apr 1970	Oct 1973	Partial for 39 mos	Subsequent methyl-CCNU maintenance

APPENDIX.—Patient population—Continued

Patient No.	Age/ sec	Prior therapy	Metastatic involvement	Onset of symptoms	Date of diagnosis	Metastases developed	Adriamycin started	Date of death	Response	Comments
5	40/M	Total thyroidectomy; radical neck dissection; ^{131}I;radiotherapy;multiple drug therapy	Pulmonary; hepatic	May 1967	May 1967	May 1969	Apr 1970	Apr 1970	Increasing disease	Died 3 days after start of adriamycin
28	51/M	Subtotal thyroidectomy; radical neck dissection; radiotherapy; methyl-CCNU	Bone; pulmonary	June 1965	Apr 1967	June 1972	Mar 1973	Oct 1974	No change	Cessation of diarrhea; failed on asaley, piperazinedione, and immunotherapy
30	43/M	Total thyroidectomy; radical neck dissection	Pulmonary	Jan 1971	Feb 1971	Oct 1972	Nov 1972	Living	Partial for 21+ mos	Still on therapy
39	56/M	Subtotal thyroidectomy; radiotherapy	Bone;hepatic	May 1969	Aug 1969	May 1973	Dec 1973	Living	No change	Cessation of diarrhea; now on methyl-CCNU
Unclassified										
7	64/M	Total thyroidectomy; radiotherapy; 5-FU;vincristine	Pulmonary	May 1969	Sept 1969	Apr 1970	Apr 1971	Jan 1972	Partial for 6 mos	Failed on subsequent methyl-CCNU
Hürthle cell										
6	54/F	Total thyroidectomy; radiotherapy	Bone; pulmonary	June 1959	Sept 1959	Nov 1965	Feb 1971	Nov 1971	Near complete remission for 7 mos	Death probably due to adriamycin-induced cardiomyopathy
19	49/F	Total thyroidectomy; ^{131}I;radiotherapy	Bone; pulmonary	Oct 1969	Jan 1970	Jan 1971	Mar 1972	Living	No change	Stabilization of previously progressing disease; on methyl-CCNU and asaley
24	54/F	Total thyroidectomy; radical neck dissection; radiotherapy	Soft tissue; bone	June 1970	June 1970	June 1972	Feb 1973	Living	Partial for 9 mos	—

27	46/M	Total thyroidectomy; radiotherapy	Bone; hepatic	June 1970	July 1970	Dec 1970	Nov 1972	Apr 1973	No change	Improved liver scan, but symptomatically worse
29	65/F	Subtotal thyroidectomy; ^{131}I;radiotherapy	Soft tissue; bone	Feb 1971	Mar 1971	Feb 1972	Jan 1973	Oct 1973	No change	Dramatic pain relief
31	72/F	Total thyroidectomy; radical neck dissection; radiotherapy	Soft tissue; pulmonary	June 1969	July 1969	Apr 1973	June 1973	Living	Near complete remission for 16+ mos	Impressive loss of dysphagia
37	48/F	Total thyroidectomy; radical neck dissection	Soft tissue; pulmonary	Feb 1971	June 1970	Jan 1974	Mar 1974	Living	Increasing disease	—
40	75/M	Total thyroidectomy; radical neck dissection; radiotherapy	Soft tissue	Dec 1963	Dec 1963	Jan 1974	May 1974	Sept 1974	No change	—
43	53/F	Total thyroidectomy; radical neck dissection; ^{131}I;radiotherapy; cyclophosphamide	Soft tissue; bone; pulmonary	June 1967	Aug 1967	Dec 1973	Apr 1974	Living	Increasing disease	Now on Baker's antifol and immunotherapy

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
Asaley: NSC-167780; CAS reg. No. 3577-89-7
Baker's antifol: NSC-139105; CAS reg. No. 41191-04-2; ethanesulfonic acid, compd. with α-[2-chloro-4-(4,6-diamino-2,2-dimethyl-*s*-triazin-1(2*H*)-yl) phenoxy]-*N*,*N*-dimethyl-*m*-toluamide (1:1); triazinate
Bleomycin: NSC-125066
***cis*-Dichlorodiammineplatinum(II):** NSC-119875; CAS reg. No. 15663-27-1
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU
MER: NSC-143769; methanol extraction residue of BCG
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[p-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-
Piperazinedione: NSC-135758; CAS reg. No. 41109-80-2; 2,5-piperazinedione, 3,6-bis(5-chloro-2-piperidyl)-, dihydrochloride
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Adriamycin (NSC-123127) Activity in Genitourinary and Gynecologic Malignancies[1]

Milan Slavik[2,3]

The genitourinary and gynecologic malignancies represent two groups of tumors in whieh adriamycin has been studied and has shown activity. This presentation will be limited to adriamycin activity against eight tumors, ie, renal cell carcinoma, bladder cancer, prostatic cancer, testicular tumors, ovarian cancer, carcinoma of the corpus uteri, cervical cancer, and vaginal cancer. The data are from both the published literature and the unpublished results reported to the Investigational Drug Branch, Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute. Although this review includes most of the available studies, it cannot be considered to encompass all of the studies dealing with these tumors. A breakdown of responses to adriamycin in the various tumor types is shown in table 1, and a comparison of this activity with that of a variety of other agents is shown in table 2.

GENITOURINARY TUMORS

Renal cell carcinoma exhibits constant resistance to adriamycin therapy (1-3),[4-7] and is also resistant to a variety of other agents.[8] On the other hand, bladder cancer shows a much higher level of responsiveness (1-3).[4-7] The overall response rate (27%) in 136 patients, including three complete remissions and a duration of remission as high as 11+ months, ranks adriamycin among the top agents against this tumor.[8] Because of its clearly established activity as a single agent, adriamycin has been used in combination with other active agents in bladder cancer as illustrated by the Southeastern Cancer Study Group protocol shown in figure 1.[5]

In adenocarcinoma of the prostate (2,3),[4-7,9] the overall response rate is 19% in 52 patients. There were no complete responses and the duration of partial remissions ranged from 1.5 to 4.5 months. Most of the data used in the comparison of adriamycin to other single agents are from broad phase II studies,[8] and results of disease-oriented phase II studies are expected to provide more detailed information on activity and cross-resistance with other drugs reported active in this disease. An example of such an approach is shown in the Eastern Cooperative Oncology Group protocol shown in figure 2.[9]

In testicular tumors there was an overall 29% response rate, with one complete remission and a median duration of remission ranging from 1 to 7 months (1,3).[4-7] Three of the studies reported responses by cell type and these are summarized in table 3 (3).[4,5] In addition to adriamycin, there are at least five other drugs with established activity against testicular tumors[8] and the logical step is to employ adriamycin in combination with these agents (fig 3).[7]

GYNECOLOGIC MALIGNANCIES

The results of adriamycin therapy in ovarian cancer (4)[4-7,10] clearly show that the high response rates obtained in studies with small numbers of patients have not been confirmed in larger investigations by the Southwest Oncology Group and by Smith. A breakdown of findings by cell type from three studies is available in table 3 (3,4)[5] which shows activity only in serous cystadenocarcinoma and malignant teratoma. Although the patient numbers are small, these results may indicate differences in activity.

Ovarian cancer is considered to be sensitive to a number of chemotherapeutic agents.[8] Because of the difference in the results obtained thus far, further phase II studies on adriamycin in ovarian cancer, such as the one outlined in figure 4,[8] are indicated.

There are hints of activity against carcinoma of the corpus uteri (3).[6] In comparison to other agents adriamycin is in a leading position in spite of the scanty amount of data compiled.[8] Phase II studies, such as the example shown in figure 5,[9] are needed. Impressive results have also been reported with adriamycin plus cyclophosphamide in endometrial cancer (fig 6).[11]

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[1]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

[2]Investigational Drug Branch, Cancer Therapy Evaluation (CTE), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

[3]*Reprint requests to:* Dr. Milan Slavik, Chief, Investigational Drug Branch, DCT, NCI, Bldg 37, Rm 6E26, National Institutes of Health, Bethesda, Md 20014.

[4]Bonadonna G, Beretta G, Doci R, et al. Evaluation of adriamycin alone and in combination in human neoplasia. Presented at the 8th International Congress of Chemotherapy, Athens, Greece, Sept 8-14, 1973.

[5]Southwest Oncology Group. Unpublished data with permission of the group chairman, 1973-1974.

[6]Central Oncology Group. Unpublished data with permission of the group chairman.

[7]Western Cancer Study Group. Unpublished data with permission of the group chairman.

[8]Investigational Drug Branch, CTE, DCT, NCI. Cumulative data.

[9]Eastern Cooperative Oncology Group. Unpublished data with permission of the group chairman.

[10]Smith J. Personal communication, 1974.

[11]Muggia F. Personal communication, 1974.

TABLE 1.—Adriamycin activity in various types of genitourinary and gynecologic cancer

Disease	Investigators*									
	Middleman et al (ref 1)	Bonadonna et al (footnote 4)	Tan et al (ref 2)	O'Bryan et al (ref 3)	SWOG protocol No. 7206 (footnote 5)	COG protocol No. 7121 (footnote 6)	WCG protocol No. 816 (footnote 7)	ECOG protocol No. 2373 (footnote 9)	Barlow et al (ref 4, footnote 12)	Smith (footnote 11)
Renal cell carcinoma										
No. evaluated	3	2	4	15	34	29	7			
No. of responses	0	0	0	0	2	2	1 CR†			
Response rate (%)	0	0	0	0	6	6	14			
Duration (mos)	—	—	—	—	3	1-12	5			
Bladder cancer										
No. evaluated	7	8	5	39	50	10	17			
No. of responses	4 (3 CR)	3	0	14	8	5	3			
Response rate (%)	57	37	0	36	16	50	18			
Duration (mos)	3-11+	4	—	3.7	3+-4.7	1-6+	2			
Prostatic cancer										
No. evaluated		1	2	9	30	2	4	4		
No. of responses		0	0	2	4	1	1	2		
Response rate (%)		—	—	22	13	50	25	50		
Duration (mos)		—	—	—	2.5+-4.5	—	1.5	—		
Testicular tumors										
No. evaluated	2	23		16	7	3	8			
No. of responses	0	10 (1 CR)		5	1	0	1			
Response rate (%)	0	43		31	14	0	12			
Duration (mos)	—	7		—	2	—	1			
Ovarian cancer										
No. evaluated		14		19	44	5	9		10	37
No. of responses		4 (1 CR)		2	4	2	0		4	4
Response rate (%)		28		11	9	40	0		40	11
Duration (mos)		6.5		—	3.3-4	—	—		—	—
Corpus uteri carcinoma										
No. evaluated				8		6				
No. of responses				3		2				
Response rate (%)				37		33				
Duration (mos)				—		2-3				

Cervical cancer						
No. evaluated	2	4	6	2	6	5
No. of responses	1	0	2	0	0	2 (1 CR)
Response rate (%)	50	0	33	0	0	40
Duration (mos)	1	—	—	—	—	1-6
Vaginal cancer						
No. evaluated						4
No. of responses						3 CR
Response rate (%)						75
Duration (mos)						6

*SWOG = Southwest Oncology Group; COG = Central Oncology Group; WCG = Western Cooperative Group; ECOG = Eastern Cooperative Oncology Group.
†CR = complete remission.

TABLE 2.—Adriamycin activity compared to other single agents in various types of genitourinary and gynecologic cancers

	Type of cancer*													
	Renal cell		Bladder		Prostatic		Testicular		Ovarian		Corpus uteri		Cervical	
Drug	R/EP	Response (%)	R/EP	Response (%)	R/EP	Response (%)	R/EP	Response (%)	R/EP	Response (%)	R/EP	Response (%)	R/EP	Response (%)
Actinomycin D							16/31	52						
Adriamycin	5/94	5	37/136	27	10/52	19	17/59	29	20/138	14	5/14	36	5/25	20
BCNU					2/15	14	2/8		2/34	6			1/7	16
Bleomycin	1/7						24/57	42	0/12				8/78	10
Busulfan					1/16	6								
CCNU	4/59	7							7/22	32			1/5	
Chlorambucil	2/15		0/10				6/8		200/390	51	0/11		11/44	25
Cyclophosphamide	2/12		2/10		8/57	14	2/2		115/262	44	4/16	25	36/187	19
Cytembena											10/30	33		
Dibromodulcitol	4/19	21	0/4		1/3		0/1		1/8					
5-Fluorouracil	3/40	8	26/74	35	19/66	29	4/15		32/101	32	7/34	20	37/159	23
Hexamethylmelamine	1/17	6	3/10		2/6		2/6		21/54	39	2/8	25	11/40	28
Hydroxyurea	7/25	28	5/8				2/6						2/13	15
Mechlorethamine					12/31	39			25/81	31			0/12	
Melphalan							49/86	57	235/494	47			4/20	20
Methotrexate			4/11		1/3		4/10		4/16	25			5/24	21
6-Mercaptopurine	1/15										0/10			
Methyl-CCNU	0/21				2/19	11	0/4		0/18				4/24	17
Mithramycin					2/36	6	155/431	36						
Mitomycin C	4/36	11	13/51	25	1/8								4/18	22
NSC-122819 (VM-26)	0/5		6/31	19	0/1		0/1		0/2		0/2		0/7	
Procarbazine			1/3				1/3							
Streptozotocin	0/10						0/2		0/9					
Vinblastine	1/12				1/2		17/25	52	3/20					
Vincristine					2/22	9	4/7		0/17				10/34	29

*R/EP = No. of responses/No. of evaluable patients.

The findings in 25 patients with cervical cancer (2-4)[4,6,7] show a 20% overall response rate, with one complete remission and a remission duration ranging from 1 to 6 months. The activity of adriamycin is comparable to other single agents in this disease.[12] Adriamycin has been employed in combination chemotherapy as illustrated in figure 7.[12]

Impressive activity in vaginal cancer has been reported by Barlow[13] who obtained three complete remissions among four evaluable patients and a median duration of remission of 6 months.

[12] See footnote 8.
[13] Barlow J. Personal communication, 1974.

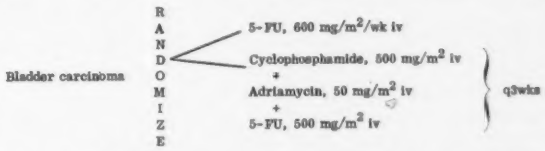
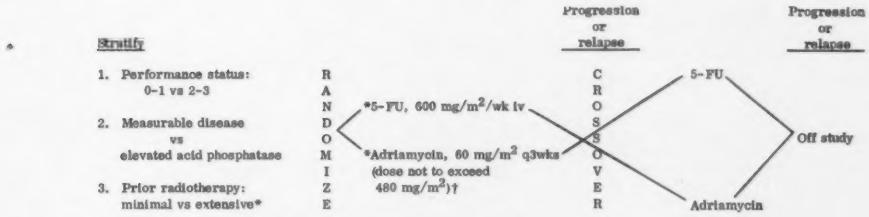
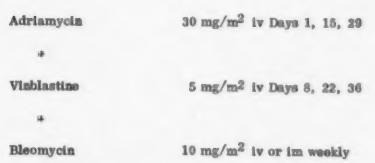


FIGURE 1.—Southeastern Cancer Study Group protocol No. 262 for bladder carcinoma.



*Doses modified for patients with extensive prior radiotherapy.
†Patients in remission receiving adriamycin therapy at 24 wks are to be switched to 5-FU maintenance at that point.

FIGURE 2.—Eastern Cooperative Oncology Group protocol No. 2373 for advanced prostatic carcinoma.



No. of patients entered: 10
No. of patients responding: 2 CR (both embryonal cell)

FIGURE 3.—Western Cancer Study Group protocol No. 145 for testicular tumors.

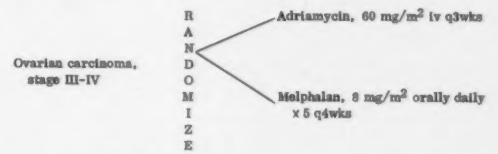


FIGURE 4.—Southeastern Cancer Study Group protocol for ovarian carcinoma.

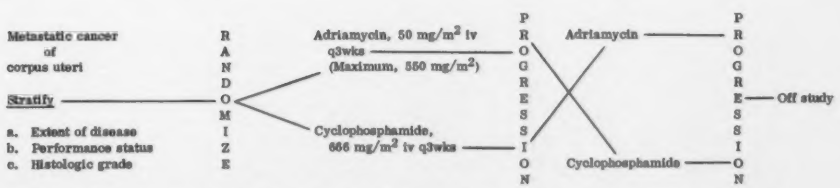


FIGURE 5.—Eastern Cooperative Oncology Group protocol No. 1173 for metastatic carcinoma of the corpus uteri.

TABLE 3.—Adriamycin activity in testicular and ovarian tumors by cell type

Cell type	No. of patients evaluated	No. of patients responding*	Response rate (%)	Duration of remission (mos)
Testicular tumors				
Seminoma	10	5	50	2-6
Embryonal	20	4	20	2
Teratocarcinoma	12	4	33	8
Choriocarcinoma	2	2 (1 CR)	100	18
Totals	44	15 (1 CR)	34	2-18
Ovarian tumors				
Serous cystadenocarcinoma	29	5	17	
Mucous cystadenocarcinoma	1	0	0	
Endometroid carcinoma	3	0	0	
Poorly differentiated	3	0	0	
Mesonephroma	1	0	0	
Granulosa theca cell	2	0	0	
Malignant teratoma	4	2	50	
Totals	43	7	16	

*CR = complete remission.

TABLE 4.—Adriamycin activity in genitourinary and gynecologic tumors

Tumor site	No. of patients evaluated	No. of patients responding*	Response rate (%)	Duration of remission (mos)
Kidney	94	5 (1 CR)	5	1-12
Bladder	136	37 (3 CR)	27	1-11+
Prostate	52	10	19	1.5-4.5
Testis	59	17 (1 CR)	29	1-7
Ovary	138	20 (1 CR)	14	3.3-6.5
Corpus uteri	14	5	36	2-3
Cervix	25	5 (1 CR)	20	1-6
Vagina	4	3 CR	75	6
Totals	522	102 (10 CR)	20	1-12

*CR = complete remission.

DISCUSSION

The overall activity of adriamycin as a single agent in genitourinary and gynecologic tumors is summarized in table 4.[14]

The data indicate that the drug is inactive in renal cell carcinoma but has established activity in bladder cancer, where it has been studied in combination with other agents, and is active in prostatic and testicular tumors.

Adriamycin efficacy in ovarian cancer remains to be further studied and disease-oriented phase II studies are needed to confirm the impressive activity reported in carcinoma of the corpus uteri, cervix, and vagina.

The high response rates and complete remissions observed clearly indicate a future trend for integration of adriamycin in combination chemotherapy for these two groups of tumors.

[14] See footnote 8.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
L-Asparaginase: NSC-109229; CAS reg. No. 9015-68-3
5-Azacytidine: NSC-102816; CAS reg. No. 320-67-2; s-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-
BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-
Bleomycin: NSC-125066
Busulfan: NSC-750; CAS reg. No. 55-98-1; 14-butanediol, dimethanesulfonate
CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
Chlorambucil: NSC-3088; CAS reg. No. 305-03-3; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Cytembena: NSC-104801; CAS reg. No. 16170-75-5; acrylic acid, 3-*p*-anisoyl-3-bromo-, sodium salt

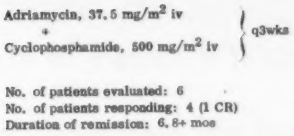

Adriamycin, 37.5 mg/m² iv
+
Cyclophosphamide, 500 mg/m² iv
q3wks
No. of patients evaluated: 6
No. of patients responding: 4 (1 CR)
Duration of remission: 6, 8+ mos

FIGURE 6.—Adriamycin plus cyclophosphamide in carcinoma of the corpus uteri.

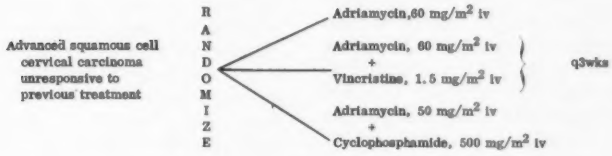


FIGURE 7.—Gynecologic Oncology Group protocol No. 23 for cervical carcinoma.

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6; daunomycin
Dibromodulcitol: NSC-104800; CAS reg. No. 10318-26-0; galactitol, 1,6-dibromo-1,6-dideoxy-
Dibromomannitol: NSC-94100; CAS reg. No. 488-41-5; 1,6-dibromo-1,6-dideoxy-D-mannitol
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU
Hexamethylmelamine: NSC-13875; CAS reg. No. 645-05-6
Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1
Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard
Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-
6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate; 6-MP
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-
Mithramycin: NSC-24559; CAS reg. No. 18378-89-7
Mitomycin C: NSC-26980; CAS reg. No. 50-07-7
NSC-45388: CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-[3,3-dimethyl-1-triazeno)-; DTIC
NSC-122819: 4'-demethylepipodophyllotoxin, 9-(4,6-0-2-thenylidene-β-D-glucopyranoside); VM-26
Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride
Streptozotocin: NSC-85998
6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-
Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Adriamycin (NSC-123127) in 87 Patients With Osteosarcoma[1,2,3]

Engracio P. Cortes,[4] James F. Holland,[5] Jaw J. Wang,[6] and Oliver Glidewell[7,8,9]

The 5-year survival rate of patients with osteosarcoma after operation, radiotherapy, or a combination of the two, ranges from 5% to 23% in 17 series (1). Radiologic evidence of pulmonary metastases occurs at a median of 8.5 months after potentially curative surgical amputation (2). These data indicate that pulmonary involvement must either have been present at the time of operation or have arisen from osteosarcoma cells that circulated in the blood during the amputation (3). The patient usually dies within 6 months from the onset of detectable pulmonary metastases (4). The effectiveness of chemotherapy in metastatic osteosarcoma is limited (1,5).

Adriamycin, a cytotoxic antibiotic derived from *Streptomyces peucetius* var. *caesius*, has been found to be active in a wide spectrum of neoplastic diseases (6). We initially reported the activity of adriamycin in osteosarcoma that had metastasized to the lungs in a small series of patients (7,8). Encouraged by this result in metastatic osteosarcoma treated with adriamycin, the Acute Leukemia Group B (ALGB) recently reported the preliminary results of a study using intermittent adriamycin treatment shortly after radical surgical amputation of primary osteosarcoma in an effort to apply chemotherapy when the body burden of tumor cells was lowest (9).

The present paper reports *(a)* the activity of adriamycin alone in the patients with metastatic osteosarcoma in our initial studies, *(b)* the activity of adriamycin in metastatic osteosarcoma as part of a phase II study of childhood solid tumors by the ALGB, and *(c)* more extensive data on amputation and adriamycin in a study of primary osteosarcoma undertaken by the ALGB.

[1]Supported by Public Health Service grants CA-11028-0751 and CA-07918 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[2]This study was conducted by the Acute Leukemia Group B (ALGB).

[3]Adriamycin: CAS reg. No. 23214-92-8; doxorubicin; ADM. Supplied by Farmitalia, Milan, Italy, through Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

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[6]Roswell Park Memorial Institute, Buffalo, NY.

[7]Operations Office, ALGB, Scarsdale, NY.

[8]We are indebted to Dr. Lowell E. Irwin, of the University of Southern California School of Medicine, and Drs. Ruth Heyn and Roxie Holland, of the University of Michigan, for joining with ALGB investigators in conducting this study.

[9]*Reprint requests to:* Engracio P. Cortes, MD, Queens Hospital Center Affiliation of Long Island Jewish Medical Center, 82-68 164th St, Jamaica, NY 11432.

METHODS AND MATERIALS

The criteria for entry in this study included histologically proved osteosarcoma, characterized by osteoid or bone apposed to tumor cells. No effort was made to subclassify osteosarcoma further into chondroblastic, fibroblastic, or osteoblastic subtypes. None of the patients had parosteal osteosarcoma, chondrosarcoma, fibrosarcoma, radiation-induced osteosarcoma, or osteosarcoma after Paget's disease, all of which have different clinical courses (10). Other criteria were a minimum leukocyte count of 4000 cells/mm^3, a platelet count of 100,000 platelets/mm^3, a blood urea nitrogen level less than 25 mg/100 ml, and a creatinine level less than 1.5 mg/100 ml. In the adjuvant study, it was also required that the primary tumor be resectable without roentgenologic evidence of metastases.

Drug Dosage and Schedule

Each vial of adriamycin was diluted with 5 ml of sterile saline and the calculated dose was injected as an intravenous bolus.

Initial Study

In a phase I study of adriamycin at the Roswell Park Memorial Institute, 17 patients with pulmonary metastases from osteosarcoma were treated with adriamycin at the following dose schedules: *(a)* 17.5 mg/m^2/day for 4 days; *(b)* 20 mg/m^2/day for 4 days; *(c)* 30 mg/m^2/day for 3 days; and *(d)* 35 mg/m^2/day for 3 days.

Selection for study was nonrandom. Each schedule was repeated at intervals of 4 weeks if hematologic toxic effects had cleared.

Phase II Study

In a phase II study of adriamycin in childhood solid tumors, 26 patients with osteosarcoma were treated at random with adriamycin at dose schedule *b* (see section on Initial Study) or *e* (15 mg/m^2/day for 4 days). Subsequently, schedule *e* was changed and random allocation to schedule *b* or *c* was undertaken.

Each schedule was repeated at intervals of 3 weeks if hematologic toxic effects had cleared.

Adjuvant Study

In the adjuvant study of amputation and adriamycin in primary osteosarcoma, adriamycin was scheduled to begin when wound healing was complete. The dose was 30 mg/m^2/day for 3 successive days, repeated every 4-6 weeks for six courses. In some patients

the first course was given in the hospital, but other treatments were administered on an ambulatory basis. All but four patients were treated within 8 weeks after the operation, and the median onset of adriamycin treatment for the entire group was 2 weeks. The total cumulative dose of adriamycin was 540 mg/m^2 over a period of 5-7 months. Thereafter, patients remained untreated.

From November 1971 to August 1974, 57 patients were entered into the adjuvant study. Thirteen patients were disqualified: seven had no records; two had gross metastases at entry; two were never started on adriamycin; and in two the treatment was stopped after two courses because the patients refused further treatment. Neither of these two latter patients had metastases at the time of discontinuation. The remaining 44 patients formed the basis of the adjuvant study.

Hematologic, biochemical, and electrocardiographic examinations, chest roentgenograms, tomography, and bone survey were conducted before, during, and after therapy to check for possible tumor recurrence and toxic effects from the drug.

The response to therapy in the inoperable or metastatic osteosarcoma was based on objective and subjective changes. The criteria for an objective response were: *(a)* complete remission—disappearance of all clinical objective and symptomatic evidence of disease; *(b)* partial remission—greater than 50% decrease in the total area of the lesion or lesions in the absence of new lesions elsewhere or tumor progression in any lesion; *(c)* improvement—tumor regression between 25% and 49%; *(d)* no change—neither improvement nor progression after two or more courses of adriamycin; and *(e)* progression—greater than 25% increase in tumor size from the original measurement or appearance of new lesions elsewhere.

The response to therapy in the adjuvant study was measured by the disease-free interval after surgical amputation. Treatment failure was defined as unequivocal roentgenologic evidence of recurrence. In this study a leukocyte count of less than 1000 cells/mm^3 required the adjustment of succeeding doses of adriamycin to 20 mg/m^2/day for 3 days. No dose adjustment was to be made if the leukocyte nadir was in excess of 1000 cells/mm^3 unless an accompanying complication such as septicemia occurred. Subsequently, the dose adjustment was changed from 20 to 25 mg/m^2.

RESULTS

Initial Study

Objective regressions of the pulmonary metastases from osteosarcoma were observed in seven of 17 patients. One had a complete remission, five had partial remissions, and one showed improvement by a 25% decrease of the tumor size. Table 1 shows the relationship between the adriamycin dose and the objective response. The higher the dose, the more likely the patient was to respond to adriamycin.

Figure 1 shows the relationship of the drug response to survival after the onset of pulmonary metastases, and the interval from the initial diagnosis to metastases. Of the 13 patients whose pulmonary metastases appeared less than 10 months after the primary diagnosis, only two patients achieved partial remissions. Of the four patients whose pulmonary metastases were discovered more than 10 months after the initial diagnosis, all attained a complete or partial remission. Furthermore, those responding patients with metastases appearing later had longer survival rates than those patients whose metastases were noted less than 10 months from the diagnosis.

Case Reports

Patient No. 1.—A 13-year-old girl had a right mid-thigh amputation because of osteosarcoma of the distal femur in June 1969. In July 1970, a solitary metastatic spherical nodule 1.5 cm in diameter was noted in the left lung (fig 2a) and adriamycin treatment was initiated at a dose of 20 mg/m^2/day for 4 days. A month later, regression of the nodule began. She was maintained on a 3- or 4-day course of adriamycin at monthly intervals. In October 1970, no appreciable lung lesion could be seen by either regular films or tomogram. Adriamycin therapy was stopped after 9 months in March 1971 when a cumulative dose of 560 mg/m^2 had been given (fig 2b). She remains free of disease over 4½ years after the start of therapy and over 3 years and 9 months without treatment.

TABLE 1.—Adriamycin dose schedules in the treatment of osteosarcoma

Dose schedule (mg/m^2)	No. of patients	No. of patients with—		
		Complete remission	Partial remission	Improvement
17.5 × 4	1			
20 × 4	5	1		
30 × 3	7		3	
35 × 3	4		2	1
Total	17	1	5	1

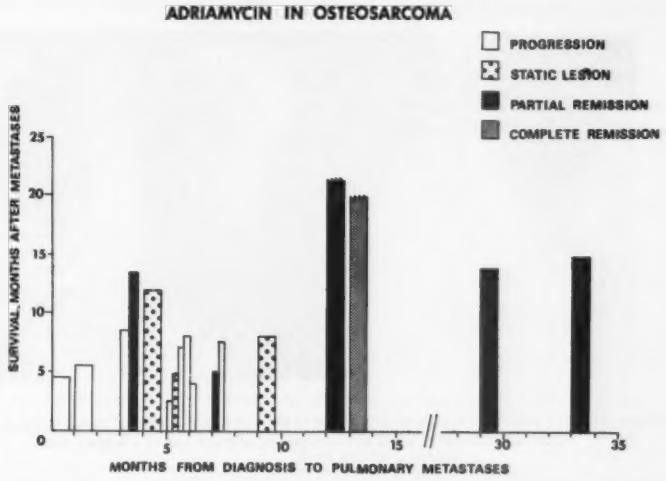

FIGURE 1.—Relationship of the drug response to survival in months after onset of pulmonary metastases, and the interval in months from the initial diagnosis to metastases.

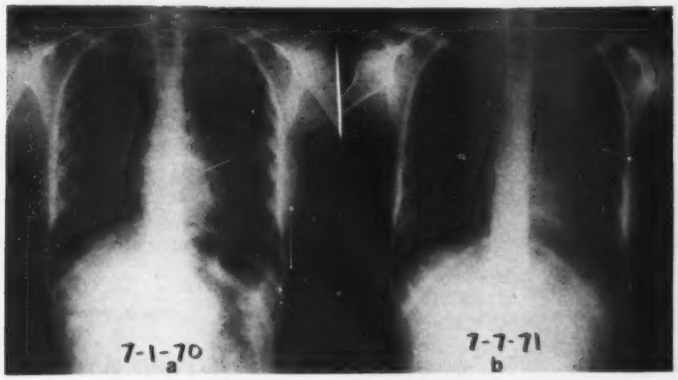

FIGURE 2.—Chest roentgenograms of patient No. 1 (a) before and (b) after repeated adriamycin therapy.

Patient No. 2.—A 16-year-old boy had left mid-thigh amputation because of osteosarcoma of the distal femur in June 1971. He was vaccinated by others with irradiated osteosarcoma tumor cells and bacillus Calmette-Guérin monthly on four occasions. In November 1971, pulmonary metastases were noted and he was given adriamycin at a dose of 20 mg/m^2/day for 3 days in December 1971. Rapid growth of the tumor continued (fig 3a). A second course of adriamycin (30 mg/m^2/day × 3) was given in January 1972 and was repeated 18 days later. This resulted in a marked regression of the tumor 7 weeks after the initiation of the higher dose (fig 3b). No further adriamycin was given because of abnormalities in liver function. He succumbed to tumor progression 1½ months after the last course of treatment.

Patient No. 3.—A 17-year-old boy had a right hip disarticulation because of osteosarcoma of the distal femur in June 1970. Three months later three pulmonary masses in the right lung were noted (only one on the lateral film). Courses of adriamycin treatment (35 mg/m^2/day × 3) were started in October 1970, which resulted in rapid disappearance of the two smaller nodules and delayed regression of the larger mass a month after the first course of therapy (figs 4a and 4b). A hypothetical total regression curve (fig 5) indicated that a very long course of chemotherapy probably lay ahead, even assuming

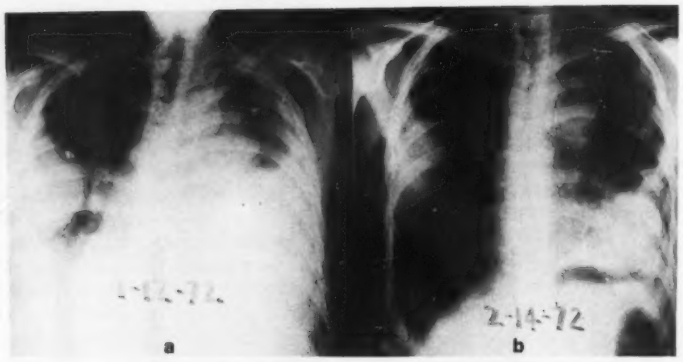

FIGURE 3.—(a) Chest roentgenogram of patient No. 2 showing tumor progression after a low dose of adriamycin; (b) chest roentgenogram more than a month later after two courses of adriamycin at a higher dose.

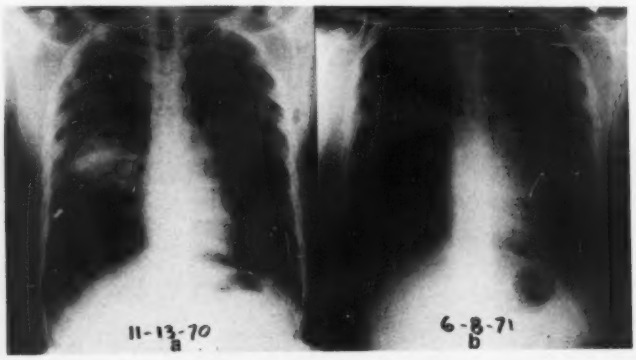

FIGURE 4.—Chest roentgenograms of patient No. 3 (a) before and (b) after adriamycin therapy.

cumulative drug toxicity and tumor resistance would not occur. Pneumonectomy was therefore performed 9 months after the initial adriamycin treatment. The lung specimen revealed a residual solitary spherical mass 3 cm in diameter. The two smaller lesions seen on the initial chest roentgenograms were not found. Another course of adriamycin was given. Three and one-half months after pneumonectomy, he had a sudden onset of right hemiparesis and dysphasia and he died 2 weeks after admission. Autopsy revealed a dilated heart weighing 450 g with a ventricular mural thrombus. Light microscopy of the myocardial muscle revealed interstitial edema. Brain sections showed left cerebral emboli. No evidence of tumor was found. He received a total cumulative dose of 1125 mg/m^2 of adriamycin in a 12.5-month period.

Patient No. 4.—A 16-year-old girl had left hip disarticulation because of osteosarcoma of the distal femur in May 1969. A year later she was given actinomycin D and cyclophosphamide because of pulmonary metastases, attaining a partial remission.[10] In March 1971 her tumor began to grow and did not respond to vincristine.[11] In October 1971 adriamycin was initiated at a dose of 30 mg/m^2 for 3 days resulting in partial tumor regression on repeated monthly 3-day courses (figs 6a and 6b). Her pulmonary lesion reached a plateau after four courses of therapy, and the drug was discontinued. A wedge resection of her pulmonary lesion was performed in March 1972 and she remains free of disease for over 2 years and 9 months without treatment.

[10]Actinomycin D: NSC-3053.

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

[11]Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

TABLE 2.—Adriamycin in the treatment of osteosarcoma: various unresectable and metastatic sites

Sites	No. of patients	No. of patients with— Partial remission	Progression	No evidence of disease
Pulmonary	13	1	12	
Bone	4	0	4	
Soft tissue	1	1		
Undetermined	4		4	
Metastatic lesion resected prior to adriamycin				
Pulmonary	3		1	2
Inguinal node	1		1	
Total	26	2	22	2

TABLE 3.—Adriamycin in the treatment of osteosarcoma in relation to the interval from primary diagnosis to pulmonary metastases

Dose schedule (mg/m^2)	No. of patients*	No. of patients with— Partial remission	Progression	Interval (mos)
15 × 4	1		1	1
20 × 4	7 (3)		7	1-9
30 × 3	5 (1)	1	4	3-9
Total	13 (4)	1	12	

*Nos. in parentheses = patients receiving only one course of adriamycin.

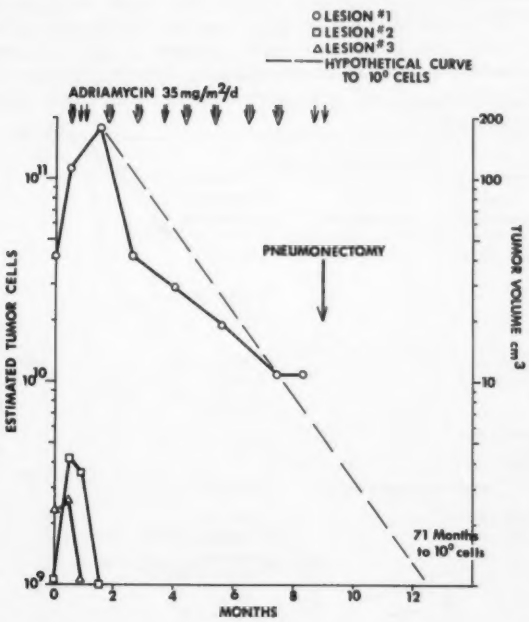

FIGURE 5.—Tumor volume of three unilateral pulmonary metastases of osteosarcoma measured by serial roentgenographic examination during adriamycin treatment. The volumes are transformed to numbers by assuming 10^9 cells/cm^3. The hypothetical regression line is shown. *See* text (patient No. 3).

Phase II Study

In the phase II study of adriamycin, 26 patients with inoperable osteosarcoma were entered. Table 2 shows the sites of unresectable primary lesions, metastases, and their responses to adriamycin. While one of the 13 patients with pulmonary metastases responded to treatment, none of the four patients with bony lesions responded. Two of the four patients whose lesions were resected prior to the start of adriamycin had progression of tumor and two others remain free of disease. Table 3 shows the relationship between the objective response of pulmonary metastases from osteosarcoma and the dose schedule of adriamycin. The only responder in this group of patients is the one patient out of five who received adriamycin at a dose of 30 mg/m^2. Four of these 13 patients received only one course of adriamycin. Table 3 also shows the interval from primary diagnosis to discovery of pulmonary metastases. All 13 patients had their pulmonary metastases discovered less than 10 months from the primary diagnosis.

One patient who had metastases to the soft tissue (abdominal wall) achieved partial remission at a dose schedule of 30 mg/m^2 of adriamycin. The discovery of this metastasis was noted 13 months after the primary diagnosis.

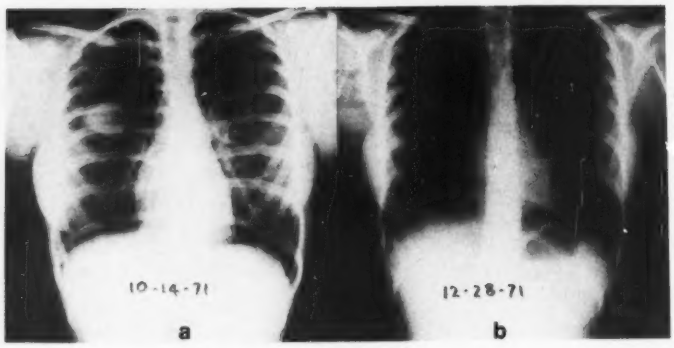

FIGURE 6.—Chest roentgenograms of patient No. 4 (a) before and (b) after adriamycin therapy.

TABLE 4.—Primary osteosarcoma and operation

Primary site	No. of patients	Operation	No. of patients
Femur			
Distal	24	**Hip disarticulation**	15
		Mid-thigh amputation	9
Middle	1	**Hip disarticulation**	1
Tibia			
Proximal	10	Above-knee amputation	10
Distal	1	Above-knee amputation	1
Fibula	2	Above-knee amputation	2
Humerus	3	Shoulder disarticulation	3
Rib	2	Total resection	2
Mandible	1	Incomplete resection	1
Total	44		44

TABLE 5.—Adriamycin in the treatment of osteosarcoma: protocol deviation and recurrence of disease

Protocol deviation	No. of patients	No. of patients with*— Lung metastases	Local recurrence	No evidence of disease
A. Adriamycin dose lowered	3	2 (6.7, 10.5)		1 (8.5+)
B. Interval between adriamycin courses >6 wks	2			2 (6.5+, 33+)
C. Subradical operation	4	1 (1.8)		3 (6.5+, 11+, 19+)
D. Combined A and B	1			1 (4.4+)
E. Combined A and C	2	2 (4.0, 4.0)		
F. Combined B and C	1	1 (14)		
G. Combined A, B, and C	3		2 (12, 18.5)	1 (7.5+)
H. None	28	3 (3.2, 7.8, 12)		25 (range = 1-38.5+, median = 6.4+, mean = 9.1+)
Total	44	9	2	33 (range = 1-38.5+, median = 6.7+, mean = 9.8+)

*Nos. in parentheses = duration in mos of disease status.

Adjuvant Study

Of the 44 patients in the adjuvant study, 26 were male and 18 were female. Their ages ranged from 8 to 60 years, with a mean and median age of 15 and 14 years respectively. Table 4 shows the primary sites of osteosarcoma and the type of surgical amputation that was done. In 27 of 44 patients (61%) the lesions were localized around the knee joint.

Pulmonary metastases are usually the first evidence of recurrent osteosarcoma. One hundred and forty-five patients less than 21 years of age with operable osteosarcomas of the extremities, reported by Marcove et al (2), were used as a historical comparison group. Only 30% were free of pulmonary metastases 18 months after operation (fig 7).

In the adriamycin-treated group, with observations up to 38.5 months, 11 of 44 patients relapsed (two of local recurrences and nine of pulmonary metastases). At 18 months, 60% of the treated patients were expected to be free of any evidence of disease, as determined by the life-table method (95% confidence limits, 39%-81%) (fig 7).

Table 5 shows the relationship of protocol deviation and recurrence of disease. For reasons unrelated to leukopenia (counts < 1000 cells/mm^3) or to delay in the leukocyte count recovery, ten patients had their adriamycin dose decreased or the interval between courses increased beyond the prescribed 6 weeks. Seven of these ten patients have relapsed. Of 16 patients whose adriamycin dose had been lowered, whose interval between courses was increased to greater than 6 weeks, whose operation had been subradical, or any combination of the above deviations, eight patients have relapsed. The disease-free

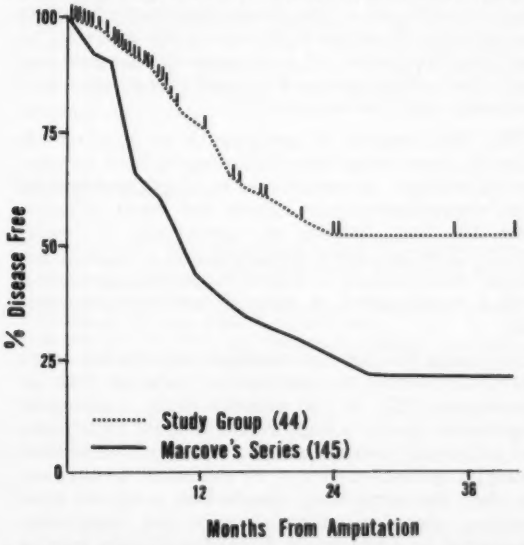

FIGURE 7.—Percentage of patients with osteosarcoma remaining free of disease after operation. Study group patients received adriamycin. Individual disease-free patients are indicated by vertical lines.

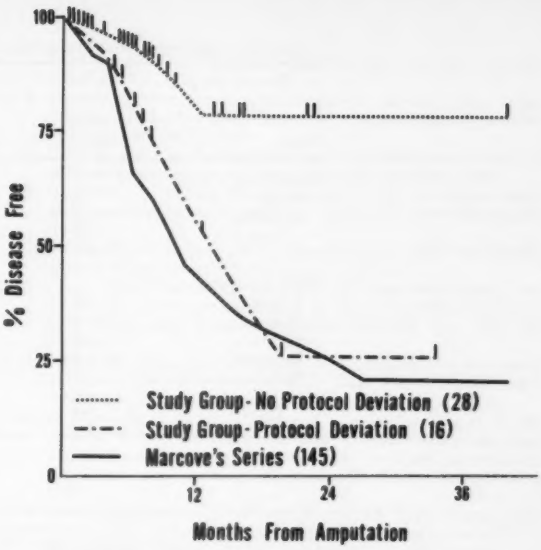

FIGURE 8.—Percentage of patients with osteosarcoma free of pulmonary metastases or local recurrence. (Vertical lines indicate individual patients free of disease.)

interval of these 16 patients at 18 months is expected to be 39% (95% confidence limits, 8%-70%) (fig 8). No protocol deviation was seen in 28 patients. In this group only three patients had pulmonary metastases, and the projected percentage of patients free of disease at 18 months is 77% (95% confidence limits, 52%-100%) (fig 8).

Table 6 shows the relation between the type of operation and the recurrence of disease. Relapses were seen in six of ten patients who had subradical amputation of the primary lesion. In contrast, pulmonary metastases developed in five of 34 patients who had radical amputation.

Nine instances of pulmonary metastases have appeared during the course of adriamycin treatment and up to 14 months after the operation. Ten patients have been observed for between 14 and 38.5 months without further emergence of pulmonary metastases. The two instances of local recurrence occurred at 12 and 18.5 months after surgery.

Adriamycin Toxic Effects

Toxic effects from adriamycin in the phase I study are shown in table 7. In this early exploration of adriamycin, fatal cardiomyopathy appeared suddenly in two patients, 10 and 12.5 months after receiving adriamycin. The drug had been given in cumulative doses of 900 and 1125 mg/m^2 respectively. The toxic effects of adriamycin in the phase II and adjuvant study were predictable and similar to those seen in the phase I study. However, no congestive heart failure was observed in these groups, since the adriamycin cumulative dose was limited to 600 mg/m^2.

TABLE 6.—Adriamycin in the treatment of osteosarcoma: surgery and prognosis

Sites	No. of patients	No. of patients with— Local recurrence	Lung metastases	No evidence of disease
Subradical surgery				
Femur	9	1	4	4
Mandible	1	1		
Total	10	2	4	4
Radical surgery				
Femur	16		3	13
Tibia	11		1	10
Fibula	2			2
Humerus	3		1	2
Ribs	2			2
Total	34	0	5	29

TABLE 7.—Toxicity of adriamycin in the treatment of advanced osteosarcoma

Toxicity	No. of patients	Percent of total patients
Alopecia	17	100
Wbc < 4000 cells/mm^3	12	70
Nausea and vomiting	11	64
Stomatitis	11	64
Hemoglobin drop > 2 g/100 ml	8	47
Platelets < 100,000 platelets/mm^3	6	35
Elevation of liver enzymes	3	18
Fatal cardiomyopathy	2	12

DISCUSSION

Based on a meager history of chemotherapeutic effectiveness in advanced osteosarcoma, it was encouraging to observe objective regression in 41% of 17 patients in the phase I study. Patients whose pulmonary metastases from osteosarcoma were discovered less than 10 months after the primary diagnosis were more refractory to treatment in the phase I and II studies. This necessitates a continued search for new chemotherapeutic agents or combination of agents for this group of patients. The refractoriness to chemotherapy in this group with rapidly appearing metastases was confirmed by Gottlieb[12] in 33 patients with osteosarcoma treated with adriamycin and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide.[13] Five of 22 (23%) of his patients responded when metastases occurred within 10 months after the initial diagnosis. In contrast, eight of 11 patients (73%) responded when metastases were noted more than 10 months after the initial diagnosis. The difference was significant at a P value < 0.01. These observations reflect the activity of adriamycin in a slowly growing tumor. The later onset of pulmonary metastases suggests that a less aggressive tumor or a greater host immunity might be responsible.

The effectiveness of adriamycin in delaying the onset of gross metastases following radical amputation of primary osteosarcoma supports the concept that chemotherapeutic agents are most effective when the body burden of tumor cells is lowest (11-13). This has been demonstrated in laboratory animal systems and in Wilms' tumor in man treated with a combination of surgery and chemotherapy (14).

For years the type of surgical amputation alone has not influenced the survival of patients with osteosarcoma (15). In the present study, subradical amputation shows a higher risk of local recurrence and pulmonary metastases (six of ten patients) than radical amputation (five of 34 patients). It is possible that the remaining unresected involved bone contains microfoci of tumor cells not completely eradicated by adriamycin. These tumor cells may be responsible for future local recurrence or pulmonary metastases (or both) upon termination of therapy.

[12]Gottlieb JA. Adriamycin in sarcoma. Presented at the New Drug Liaison meeting, DCT, NCI, Bethesda, Md, Dec 10-11, 1973.

[13]5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide: NSC-45388; CAS reg. No. 4342-03-4.

Lowering the dose of adriamycin to avoid leukopenia was also associated with recurrence of disease. This dose-dependent antitumor activity of adriamycin in osteosarcoma is consistent with our phase I results.

The toxic effects from adriamycin have been well tolerated. The cardiomyopathy in patients receiving a total cumulative dose of adriamycin greater than 550 mg/m^2 (16,17) as seen in two of 17 patients in the phase I study was not seen in any of the 70 patients in the phase II or adjuvant study, when the total cumulative dose of adriamycin was limited to less than 600 mg/m^2. The present report indicates that adriamycin is effective in metastatic osteosarcoma when given at 30-35 mg/m^2/day for 3 days repeated at 4-week intervals and if given to patients whose pulmonary metastases first became apparent more than 10 months after the primary diagnosis.

The administration of adriamycin following radical amputation of the primary osteosarcoma is highly effective in delaying the clinical appearance of pulmonary metastases and death. Of patients treated precisely in accordance with the study protocol, 25 of 28 patients (89%) showed no evidence of metastases or recurrence. In contrast, only eight of 16 patients without evidence of metastases or relapse when infractions of the protocol occurred which decreased the dose of adriamycin, decreased the radical nature of the surgery, or lengthened the intervals between treatment. Whether this form of adjunctive chemotherapy for osteosarcoma will result in an augmented cure rate remains to be proved. Application of this treatment in practice should be conducted in association with research centers where data can be accumulated to define the eventual optimal treatment for osteosarcoma. The simultaneous construction of other effective chemotherapies for osteosarcoma suggests a basis for optimism concerning the hitherto bleak prognosis for this neoplasm (18-22).

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Combination Chemotherapy With Adriamycin (NSC-123127) in Primary Treatment of Osteogenic Sarcoma[1,2]

Wataru W. Sutow[3,4]

SUMMARY

Adriamycin was utilized as one component of a four-drug combination chemotherapy regimen along with vincristine, cyclophosphamide, and melphalan (conpadri) in the primary treatment of osteogenic sarcoma after surgical amputation. Of 43 patients treated, 24 (55%) have remained free of disease from 2 to 49 months from the time of amputation. Nineteen of those 24 patients have been observed for more than 12 months and 13 have been observed for more than 24 months.

[Cancer Chemother Rep Part 3, vol 6:315-317, 1975]

Adriamycin was administered by investigators of the Southwest Oncology Group (SWOG) as one component of a multimodal, multidrug approach to the primary management of patients with nonmetastatic osteogenic sarcoma.[4] The development of the multidrug regimen was the result of systematic phase II studies of various drugs and drug combinations in patients with metastatic cancer and subsequently in patients with nonmetastatic cancer.

Clinical investigations, beginning in 1962, indicated that an alkylating agent, melphalan, produced sporadic but sometimes impressive regressions of metastatic osteogenic sarcoma (1). The use of melphalan in 14 patients with nonmetastatic cancer between 1962 and 1967, however, did not significantly improve the cure rate (2).

About 1966, the consistent efficacy of a three-drug combination of vincristine, actinomycin D, and cyclophosphamide (VAC) was demonstrated in the treatment of childhood rhabdomyosarcoma (3). In 1968, the improved antitumor effect of cyclophosphamide, given in high-dose intermittent pulses, was noted (4). Furthermore, single-drug investigations also indicated that cyclophosphamide occasionally produced regression of metastases from osteogenic sarcoma (5). Consequently, beginning in 1968, we utilized a pulse-VAC regimen in association with surgical amputation in the primary treatment of osteogenic sarcoma. Eleven children were treated. Three have remained free of disease for more than 5 years from the time of amputation, a survival rate of 29%,[5] which is somewhat better than the usual clinical experience with this tumor (6).

About 1970, early indications were that adriamycin was capable of producing regression of metastatic osteogenic sarcoma in a number of patients (7,8). Based on the results of these phase I and II studies, a four-drug regimen (conpadri) was developed which incorporated adriamycin into a modification of the melphalan-VAC schedules (8). Treatment of the first patient was started in March 1971. This report summarizes the SWOG experience with the conpadri regimen in the treatment of 43 patients with osteogenic sarcoma.

CONPADRI SERIES

All of the patients had surgical amputation for osteogenic sarcoma. None had any evidence of metastatic disease when chemotherapy was started. All had a definitive diagnosis of osteogenic sarcoma; no patient with chondrosarcoma or with sarcomas other than osteogenic sarcoma was included. No cases of parosteal osteogenic sarcoma were present in the patient population. One patient in the study group had thoracic surgery and extirpation of metastatic disease prior to admission to the present study. In most cases, chemotherapy was started as soon after the amputation procedure as wound healing was considered to be satisfactory.

The sequences and time intervals between drug courses are indicated schematically in figure 1 (9).

In the absence of metastases, chemotherapy was continued over a period of 72 weeks, after which all drug treatment was discontinued.

The doses of adriamycin for the various pulses as well as the cumulative total dose of the drug with respect to time are shown in table 1. The first six pulses of adriamycin were given as single intravenous (iv) push doses of 1.5 mg/kg (45 mg/m^2) each.

[1]Supported in part by Public Health Service grants CA-02501 and CA-03713 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[2]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text. Adriamycin was supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

[3]Department of Pediatrics, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute at Houston.

[4]*Reprint requests to:* Wataru W. Sutow, MD, Department of Pediatrics, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77025.

[5]Protocol study No. SWOG-979.

[6]Sutow WW, Sullivan MP, Wilbur JR, et al. Study of adjuvant chemotherapy in osteogenic sarcoma. In preparation.

Each of the two subsequent courses were administered in two doses of 1.0 mg/kg (30 mg/m^2), given a week apart. The cumulative total dose for the entire regimen was 13 mg/kg (390 mg/m^2) given over a period of 55 weeks.

RESULTS

Data are available on three consecutive series of patients who received the conpadri regimen. All 18 patients in the first series had amputations prior to July 1972. In the second series of 19 patients, amputations were performed between July 1972 and December 1973. The six patients having amputations after January 1, 1974, were included in the third series. The followup data on the three series of patients are summarized in table 2.

The patients in the first series have the longest followup period, ranging from 27 to 49 months after amputation with a median of 36 months. Ten of 18 patients (55%) are living with no evidence of disease 12-34 months from the time all chemotherapy was discontinued.

In the second group of 19 patients, the shortest followup period was 11 months and the longest was 27 months from the time of amputation. Nine of the 19 patients (47%) are living with no evidence of disease. The third group of five patients has the shortest followup experience, ranging from 2 to 10 months. When the entire group of 43 patients is combined, 24 patients (55%) are living with no signs of disease activity.

Toxicity manifestations seen in the conpadri study conformed closely to the anticipated and well-known patterns related to the various drugs. Leukopenia and marrow depression were severe after many of the cyclophosphamide courses. Because of the severity of these problems as well as nausea and vomiting associated with the administration of cyclophosphamide in the young adult patient as compared to children, a maximum single dose of 500 mg of the drug was used in some of the larger patients. Hematuria rarely occurred.

The occurrence of leukopenia after the administration of adriamycin at the dose levels used in this study was not a major problem. ECG changes that resulted in discontinuance of adriamycin as a precautionary measure were seen in two patients.

DISCUSSION

The followup periods of the survivors in the first series treated with the conpadri regimen range from 27 to 49 months with a median of 36 months. The patients have not received any further chemotherapy for 11 months or longer. On the basis of published data, it seems highly unlikely that metastases will develop in those remaining disease-free. The data for the second series of patients parallel closely those data obtained in the first series. Thus, it appears reasonable to presume that the use of the conpadri regimen in the primary treatment of osteogenic sarcoma results in disease-free survival in slightly more than 50% of the patients. When it is observed that the survival rate for patients with this cancer for the past number of decades has been around 20% at best (6), the survival rate with conpadri represents a significant improvement.

In recent months, the results of chemotherapy in the primary treatment of osteogenic sarcoma have

TABLE 1.—Adriamycin dose schedule in conpadri regimen

Course No.	Time from start of therapy (wks)	Dose (mg/m^2)	Cumulative dose (mg/m^2)
1	0	45	45
2	3	45	90
3	6	45	135
4	18	45	180
5	21	45	225
6	24	45	270
7	36	60	330
8	54	60	390

TABLE 2.—Current status of patients receiving conpadri regimen

	First series	Second series	Third series	Total
No. of patients	18	19	6	43
No. of patients free of disease	10	9	5	24
Percent of patients free of disease	55	47	83	55
Observation period				
Range (mos)	27+-49+	11+-27+	2+-10+	2+-49+
Median (mos)	36+	19+	7+	

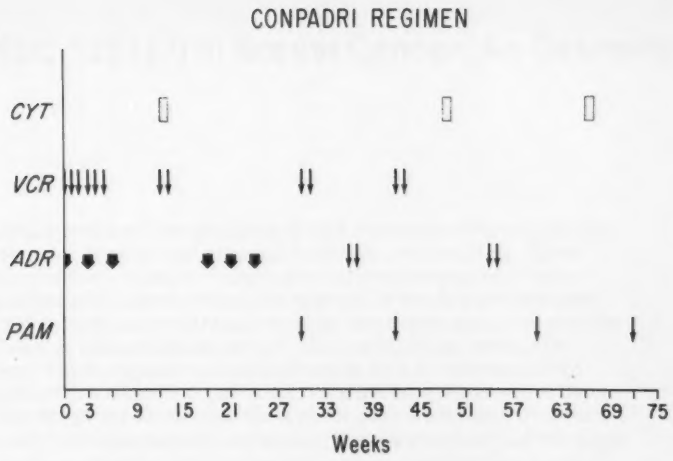

FIGURE 1.—Schema indicating time and sequence of drug administration in the conpadri regimen. Doses=cyclophosphamide + cyclophosphamide pulses (CYT), 10 mg/kg/day × 7 days; vincristine (VCR), 2 mg/m²/dose/wk; adriamycin (ADR) (heavy arrow), 1.5 mg/kg/dose; adriamycin (thin arrow), 1.0 mg/kg/dose; melphalan (PAM), 0.3 mg/kg/dose (see ref 9).

been reported by several groups of investigators (10, 11). Cortes et al have described a study in which adriamycin was used as a single-drug regimen. On the basis of life-table analyses, the investigators projected a 71% metastases-free rate for the 21 patients currently in the study (11). The necessity of administering an adequate dose of adriamycin was stressed by this group (7,11).

Even more impressive results were obtained after the administration of massive doses of methotrexate in conjunction with citrovorum factor (10). Nineteen of 20 patients were surviving with followup periods of 2-23 months (10). In an effort to combine the therapeutic efficacy of these several chemotherapy programs, the SWOG is currently utilizing a five-drug regimen (compadri-II) in which high-dose methotrexate courses have been added to the original four-drug conpadri regimen.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
Conpadri: cyclophosphamide, vincristine (Oncovin), melphalan (L-phenylalanine mustard), and adriamycin
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[p-bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-; L-phenylalanine mustard; L-sarcolysin; PAM
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[p-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
VAC: vincristine, actinomycin D, and cyclophosphamide
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); Oncovin

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Adriamycin (NSC-123127) in Breast Cancer: An Overview of Studies[1]

D.C. Tormey[2,3,4]

SUMMARY

As a single agent, adriamycin has been associated with cumulative response rates of 28% and 43% in previously treated and untreated patients, respectively. These response rates approximate those of many combination chemotherapy regimens; however, the duration of the adriamycin responses appears to be shorter. Adriamycin-containing combination regimens which incorporate methotrexate or vincristine do not appear to have been as successful as adriamycin combinations with other drugs, particularly those which employ cyclophosphamide with or without 5-fluorouracil. Trends emerging from clinical trials comparing adriamycin combinations to combinations of other drugs are discussed. Most of the data from these trials are preliminary and presently, the information concerning the comparability of the populations of each of the studies as well as the various definitions used by the investigators is incomplete. The final published data from these trials should help to more completely define the role of adriamycin in the treatment of breast cancer.

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Since the introduction in 1967 of the *Streptomyces peucetius* extract known as adriamycin, the drug has undergone extensive clinical trials and has demonstrated activity against a wide variety of human neoplasms. The present review was undertaken in an attempt to define the current status of the completed and ongoing trials including adriamycin in breast cancer. Many of these trials are still in progress and the data are therefore preliminary.

METHODS AND MATERIALS

The data compiled in this overview include all the breast cancer trials listed in the files of the Treatment Committee of the Breast Cancer Task Force (BCTF) on October 1, 1974. These files have input from clinical trials of the cooperative oncology groups, the DCT contracts, and the BCTF contracts. Investigations not receiving support through the above programs are also included. Most of the data from these trials are not yet published. The definitions used concerning response rates and durations are those of the investigators. Most of the investigators chose a 50% reduction in the cross-sectional products of at least one lesion as constituting a partial remission. A complete remission was generally defined as a complete elimination of all clinical evidence of disease. In those situations where a distinction is made between the number of patients receiving an "adequate trial" and the number of patients entered in a study, the latter figure is used as the denominator for calculating response rates. This assignment was chosen because the term "adequate trial" has different meanings to different investigative protocols. Response durations were generally taken as the time from initiation of therapy to the time of relapse. In a few instances this necessitated adding the "mean time to response" to the "mean time from remission to progression." On other occasions the definitions of response duration were not given and the values supplied by the authors were utilized. No attempt was made to analyze the programs for their relative side effects since the data for most trials are not yet available to the BCTF files.

RESULTS

Current Adriamycin Combinations Under Study

Table 1 depicts the current drugs which have been combined with adriamycin. Many of these combina-

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[1]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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tion regimens are still under investigation. Adriamycin has been under evaluation with the antimetabolites, 5-fluorouracil (5-FU) and methotrexate, as well as the vinca alkaloid, vincristine. Among the alkylating agents it has been tested with are: cyclophosphamide, dibromodulcitol, CCNU, and methyl-CCNU. It has not been investigated in combination with prednisone alone. In the three-drug combination area it has been under extensive evaluation with cyclophosphamide and 5-FU and is currently under test with dibromodulcitol and vincristine. It has also been studied with vincristine and methotrexate, and vincristine and prednisone. This leaves a large number of three-drug combinations untested, eg, with cyclophosphamide and vincristine, and with 5-FU and methotrexate. Adriamycin has been under investigation in combination with cyclophosphamide, methotrexate, and 5-FU, as well as with a small number of other three-, four-, and five-drug combinations depicted in table 1.

TABLE 1.—ADR combinations under study

ADR combined with —*	Investigators†
FU	COG, SWG
VCR	MILAN, ECOG, COG, MAYO, WCG
MTX	MAYO
CYT	TUCSON, COG, MAYO/ALB/BUF
DBD	NCI
CCNU	MDA
MCCNU	BOSTON
CYT, FU	NCI, MDA, SEG, SWG, MAYO/ALB/BUF
DBD, VCR	NCI
VCR, MTX	MAYO
VCR, PRED	MILAN, EORTC
CYT, MTX, FU	KANSAS, SWG
CYT, FU, BCG	MDA
CYT, FU, PRED‡	SWG
CYT, FU, VCR, PRED	ALGB
CCNU, CYT, MTX, VCR, PRED	WRGH

*FU= 5-fluorouracil; VCR = vincristine; MTX = methotrexate; CYT = cyclophosphamide; DBD = dibromodulcitol; MCCNU = methyl-CCNU; PRED = prednisone; BCG = bacillus Calmette Guerin.

†COG = Central Oncology Group; SWG = Southwest Oncology Group; MILAN = National Cancer Institute of Milan; ECOG = Eastern Cooperative Oncology Group; MAYO = Mayo Clinic; WCG= Western Cooperative Oncology Group; TUCSON =University of Arizona; ALB =Albany Medical Center; BUF = Roswell Park Memorial Institute; BOSTON = Sidney Farber Cancer Center; MDA = M.D. Anderson Hospital and Tumor Institute; SEG = Southeastern Cooperative Oncology Group; EORTC =European Organization for Research on Treatment of Cancer; KANSAS = University of Kansas; ALGB = Acute Leukemia Group B; WRGH = Walter Reed General Hospital.

‡Does not include trials in patients with no prior chemotherapy.

Single-Agent Adriamycin Trials

Table 2 depicts the response rates observed with adriamycin in eight different trials involving patients who had received prior chemotherapeutic agents. Responses were observed in 71 of 253 (28%) patients. Complete remissions were observed in 1.5% of the patients. The response rates range from 0 to 48% giving a median response rate of 23%. The duration of the responses has been reported as 4, 4, and 7 months in three different trials.

Single-agent adriamycin trials in patients who had received no prior chemotherapy have resulted in a cumulative response rate of 43% (54 of 126) with 3% of the patients obtaining a complete remission (table 3). The response durations in these five trials were between 3+ and 7 months. There does not appear to be a strong influence of the dosage schedule utilized upon either the response rates or durations in any of the above data.

Trials Including Adriamycin in Combinations

The NCI in Milan and the ECOG (table 4) have tested adriamycin in combination with vincristine in patients not previously exposed to chemotherapy. Both studies have yielded equivalent results. There were 33 responders among 71 patients (46%) with 8% of the patients achieving a complete remission. The early estimate of the duration of the responses is from 4+ to 6+ months. The addition of prednisone to the Milan regimen did not appear to improve the results observed in the adriamycin-vincristine regimen (table 5).

These data are in marked contrast to those obtained from patients who have been previously treated with other chemotherapeutic drugs (table 6). Under these conditions there are only five responses among 28 patients (18%). The response duration in those patients who had received prior chemotherapy was 7+ months in one study and is not yet available for the other trials. It would appear that it is too early to adequately evaluate the duration of the remissions in the adriamycin-vincristine regimens. Although the complete remission rate with this two-drug regimen appears to be slightly superior to adriamycin used alone, further information concerning the pretreatment characteristics of the patients will be necessary for a full evaluation.

Adriamycin has been combined with methotrexate and with methotrexate plus vincristine in two trials at the Mayo Clinic (table 5). Responses were observed in ten of 24 (42%) patients with a duration of 8+ months with the adriamycin-methotrexate combination, whereas responses were observed in 12 of 23 patients (52%) with a duration of 7+ months with the adriamycin-methotrexate-vincristine regimen. These data do not appear to be superior to the adriamycin-vincristine data at this time. There is very little information about the use of the methotrexate-containing regimens for patients who have received

TABLE 2.—Single-agent ADR trials in patients previously exposed to chemotherapy

Study group	Dose (mg/m²)/ schedule (iv)	No. of patients evaluated	No. of patients with—		Mean duration of response (mos)
			Response (%)	Complete response (%)	
MAYO*	60/q3wks	8	0 (0)		—
SWG	60/q3wks	14	1 (7)	1 (7)	—
SWG†	60/q3wks	16	3 (19)		—
MILAN	Multiple schedules	26	6 (23)	1 (4)	4
SWG‡	25/q3wks¶	24	5 (21)		—
	50/q3wks¶	28	3 (11)		—
	45/q3wks	19	8 (42)		—
	60/q3wks	16	2 (12)		—
	75/q3wks	33	16 (48)		—
SWG	60/q3wks	40	15 (38)	2 (5)	7
COG	0.4 mg/kg/day on Days 1, 2, 3, 8, 9, 10, then weekly	22	9 (41)		4
BCRC§	60/q3-4wks	7	3 (43)		—
Totals		253	71 (28)	4 (1.5)	4-7

*Crossover from CYT, FU, PRED.
†Crossover from nitrosoureas.
‡Randomized dose-seeking trial.
§BCRC = Baltimore Cancer Research Center.
¶ "Poor risk" patients only.

TABLE 3.—Single-agent ADR trials in patients not previously exposed to chemotherapy

Study group	Dose and schedule (iv)	No. of patients evaluated	No. of patients with—		Mean duration of response (mos)
			Response (%)	Complete response (%)	
MH*	0.4 mg/kg/day × 3, then weekly	21	9 (43)		7
MAYO	60 mg/m² q3wks	20	9 (45)		7
SWG	60 mg/m² q3wks	40	18 (45)	1 (2)	4
ECOG	60 mg/m² q3wks	13	7 (54)	3 (23)	3+
ALB/BUF	75 mg/m² q3wks	32	11 (34)		6+
Totals		126	54 (43)	4 (4)	3-7

*MH = Memorial Sloan-Kettering Hospital.

TABLE 4.—Trials with ADR-VCR combinations in patients not previously exposed to chemotherapy

Study group	Drug	Iv dose (mg/m²)	Schedule	No. of patients evaluated	No. of patients with—		Mean duration of response (mos)
					Response (%)	Complete response (%)	
MILAN	ADR	75	Day 1 q3wks	40	19 (48)	3 (8)	4+
	VCR	1.4	Day 1 × 8				
ECOG*	ADR	60	Day 1 q3wks	31	14 (45)	3 (7)	6+
	VCR	1.2	Day 1				
Totals				71	33 (46)	6 (8)	4-6+

*Ongoing trial.

TABLE 5.—Trials with ADR combinations in patients not previously exposed to chemotherapy

Study group	Drug	Dose (mg/m²)/ schedule	No. of patients— Evaluated	No. of patients— Responding (%)	Mean duration of response (mos)
MILAN	ADR VCR PRED	75/iv Day 1 q3wks 1.4/iv Days 1, 8 100/orally, Days 1-5	20	10 (50)	3+
MAYO	ADR MTX	60/iv Day 1 q5wks 15/iv Days 1, 4	24	10 (42)	8+
MAYO	ADR MTX VCR	40/iv Day 2 q5wks 10/iv Days 1, 5 1.4/iv Days 1, 5	23	12 (52)	7+
MDA	CCNU ADR	65-100/orally q6wks 40-60/iv q3wks	9	5 (56)	—
ALGB*	CYT ADR FU VCR PRED	100/orally Days 1-14 q28d 25/iv Days 1, 8 500/iv Days 1, 8 1.0/iv Days 1, 8 40/orally Days 1-14	3	2 (67)	—
WRGH	CCNU CYT ADR MTX VCR PRED	80/orally Day 1 q8wks 800/iv Days 1, 8 q4wks 15/iv Days 1,4,8,11 q4wks 20/orally Days 1, 4, 8, 11 q4wks 1.4/iv Days 1, 8 q4wks 30/orally Days 1-13 q4wks	7	3 (43)	—
ALB/BUF*	CYT FU PRED CYT ADR	150/iv Days 1-5 q5wks 300/iv Days 1-5 30 mg/day × 2 wks, then taper to 10 mg/day alternating with— 500/iv Day 1 q3-4wks 40/iv Day 1	4	3 (75)	—

*Ongoing trial.

TABLE 6.—Trials with ADR-VCR combinations in patients previously exposed to chemotherapy

Study group	Drug	Dose (mg/m²)/ schedule (iv)	No. of patients— Evaluated	No. of patients— Responding (%)	Mean duration of response (mos)
MILAN	ADR VCR	75/Day 1 q3wks 1.4/Days 1, 8	12	3 (25)	—
MAYO	ADR VCR	45/Day 3 q4wks 1.0/Days 1, 2	11	1 (9)	—
COG	ADR VCR	0.4-0.6 mg/kg/wk 0.02 mg/kg/wk	3	1 (33)	7+
ECOG*	ADR VCR	60/Day 1 q3wks 1.2/Day 1	2	0 (0)	—
Totals			28	5 (18)	7+

*Ongoing trial.

TABLE 7.—Trials with ADR combinations in patients previously exposed to chemotherapy

Study group	Drug	Dose (mg/m²)/ schedule	No. of patients— Evaluated	No. of patients— Responding (%)
MDA*	CCNU	65-100/orally q6wks	4	4 (100)
	ADR	40-60/iv q3wks		
BOSTON*	MCCNU	150-200/orally q6wks	19	3 (16)
	ADR	60-90/iv q6wks		
MAYO†	MTX	15/iv Days 1, 4 q5wks	7	0 (0)
	ADR	60/iv Day 1		
MAYO†	MTX	10/iv Days 1, 5 q5wks	2	0 (0)
	ADR	40/iv Day 2		
	VCR	1.4/iv Days 1, 5		
COG*	CYT	100 mg/day orally	2	1 (50)
	ADR	0.4 mg/kg/wk iv		
NCI‡	DBD	70+/orally Days 1-10 q4wks	31	12 (39)
	ADR	30-60/iv Day 1		
NCI§	CYT	100/orally Days 1-14 q4wks	5	4 (80)
	ADR	30/iv Days 1, 8		
	FU	500/iv Days 1,8		
MDA§	CYT	750/iv Day 1 q3wks	12	5 (42)
	ADR	50/iv Day 1		
	VCR	1.4/iv Days 1, 8		
	PRED	25 mg orally qid Days 1-5		

*Prior therapy not specified in BCTF files.
†Prior CYT, FU, and PRED combination.
‡Prior CYT, MTX, and FU combination.
§Prior drugs other than study drugs.

TABLE 8.—Trials with ADR-CYT combinations in patients not previously exposed to chemotherapy

Study group	Drug	Dose (mg/m²)/ schedule	No. of patients— Evaluated	No. of patients— Responding (%)	Mean duration of response (mos)
TUCSON*	CYT	200/orally Days 3-6 q3-4wks	55	40 (72)	9
	ADR	40/iv Day 1			
MAYO, ALB, and BUF†	CYT	500/iv Day 1 q3-4wks	TE‡	TE‡	TE‡
	ADR	40/iv Day 1			
Totals			55	40 (72)	9

*Prior FU exposure allowed in 6 patients.
†Ongoing trial.
‡Too early to evaluate.

previous chemotherapy (table 7). Table 7 also depicts the results of combining adriamycin with CCNU or methyl-CCNU in patients previously exposed to chemotherapy. There were four responses in four patients with CCNU and only three responses in 19 patients receiving methyl-CCNU.

Table 8 depicts the results of two trials combining cyclophosphamide with adriamycin. At the present time there is a 72% response rate among 55 patients in the University of Arizona trial with a mean remission duration of 9 months.

When cyclophosphamide and 5-FU are combined with adriamycin the overall response rate appears to approximate that seen with the cyclophosphamide-adriamycin regimens (table 9). The response rate is 70% among 67 patients with the response durations extending from 6+ to 10+ months.

TABLE 9.—Trials with ADR-CYT-FU combinations in patients not previously exposed to chemotherapy

Study group	Drug	Dose (mg/m²)/ schedule	No. of patients— Evaluated	Responding (%)	Duration of response (mos)
NCI	CYT	100/orally Days 1-14 q4wks	28	19 (68)	10+
	ADR	30/iv Days 1, 8			
	FU	500/iv Days 1,8			
MDA	CYT	500/iv Day 1 q3wks	39	28 (72)	6+
	ADR	50/iv Day 1			
	FU	500/iv Days 1,8			
MAYO, ALB, and BUF*	CYT	400/iv Day 1 q4wks	TE†	TE†	TE†
	ADR	40/iv Day 1			
	FU	200/iv Days 1-3			
Totals			67	47 (70)	6-10+

*Ongoing trial.
†Too early to evaluate.

TABLE 10.—Trials with ADR combinations from which data are not currently available

Study group	Drugs	Study group	Drugs
MDA	CYT + FU + BCG	EORTC	VCR + PRED
NCI	DBD + VCR	KANSAS	CYT + FU + MTX
SWG	FU	SWG	CYT + FU + PRED
	CYT + FU	COG	FU
	CYT + FU + MTX	WCG	VCR

Tables 8 and 9 show the results of trials in patients who had had no prior exposure to chemotherapy. Other results of trials in which previous exposure to chemotherapeutic agents was allowed are shown in table 7. At the NCI, an adriamycin and dibromodulcitol combination has resulted in 15 responses among 42 patients (36%) with a mean duration of 5+ months in the responders at the present time. We have also obtained four responses among five patients to a cyclophosphamide, adriamycin, and 5-FU regimen. A combination regimen employing cyclophosphamide, adriamycin, vincristine, and prednisone in patients previously exposed to other chemotherapeutic drugs resulted in five responses among 12 patients.

Other combinations which have been tested in patients who have received no prior chemotherapy are outlined in table 5. CCNU, when combined with adriamycin, has produced five responses among nine patients at the M. D. Anderson Hospital. The Acute Leukemia Group B (ALGB) has just initiated a trial substituting adriamycin for methotrexate in an intermittent "Cooper regimen." The Walter Reed General Hospital terminated a trial employing CCNU, cyclophosphamide, adriamycin, methotrexate, vincristine, and prednisone after observing only three responses among seven patients. At the Albany Medical Center and at Roswell Park Memorial Institute an interesting program employing a cyclophosphamide, 5-FU, and prednisone combination alternating each month with a cyclophosphamide and adriamycin combination has produced responses in three of the first four patients entered in the study. This is an ongoing trial, as is the ALGB trial, and further data are not yet available. Table 10 lists the drugs used in a variety of adriamycin combinations for which no results are available.

Table 11 outlines the cumulative response rates among the various regimens discussed above as a function of whether or not the patients had received previous chemotherapy. Adriamycin used alone has been associated with a 43% response rate in the absence of prior chemotherapy and a 28% response rate in the presence of prior chemotherapy. The data from the adriamycin-CCNU and adriamycin-methyl-CCNU combinations suggest that the CCNU-adriamycin combination may be the more active of the two regimens. Cyclophosphamide combined with adriamycin has been associated with a high response rate in patients who have not had previous chemotherapy. A combination of dibromodulcitol and adriamycin has been associated with a 36% response rate among patients receiving intensive prior chemotherapy. It does not appear to be advantageous to add

TABLE 11.—Cumulative response rates in regimens containing ADR

ADR combined with—	No. of patients with no prior chemotherapy—		No. of patients with prior chemotherapy—	
	Evaluated	Responding (%)	Evaluated	Responding (%)
—	126	54 (43)	253	71 (28)
CCNU	9	5 (56)	4	4 (100)
MCCNU	—	—	19	3 (16)
CYT	55	40 (72)	2	1 (50)
DBD	—	—	31	12 (39)
VCR	71	33 (46)	28	5 (18)
VCR, PRED	20	10 (50)	—	—
MTX	24	10 (42)	7	0 (0)
MTX, VCR	23	12 (52)	2	0 (0)
CYT, FU	67	47 (70)	5	4 (80)
CYT, VCR, PRED	—	—	12	5 (42)
CYT, FU, VCR, PRED	3	2 (67)	—	—
CCNU, CYT, MTX, VCR, PRED	7	3 (43)	—	—
CYT, FU, PRED alternating with CYT, ADR	TE	—	—	—

TABLE 12.—Comparative randomized trials comparing ADR to other regimens*

Study group	Drug regimens†	No. of patients—		Mean duration of response (mos)
		Evaluated	Responding (%)	
SWG	CYT, MTX, FU, VCR, PRED (C)	69	37 (54)	9
	CYT, MTX, FU, VCR, PRED (I)	67	28 (42)	11
	ADR (I)	40	18 (45)	4
	CYT, FU, PRED (I)	16	7 (44)	10+
MAYO	CYT, FU, PRED, VCR (I)	12	5 (42)	12+
	ADR (I)	20	9 (45)	7
ALB/BUF	CYT, FU, PRED (I)	45	23 (51)	10+
	Adrenalectomy	36	11 (30)	10+
	ADR (I)	32	11 (34)	6+
ECOG	CYT, MTX, FU (I)	12	4 (33)	3+
	ADR (I)	13	7 (54)	3+

*Patients with prior chemotherapy not eligible for these trials.
† C = weekly therapy; I = >13 days between courses.

prednisone to an adriamycin-vincristine regimen in the absence of prior chemotherapy. The response rate to the adriamycin-vincristine regimen dropped by about two thirds if the patients had been exposed to prior chemotherapy. At the present time the combination of adriamycin plus methotrexate or plus methotrexate and vincristine appears to be essentially equivalent to the combination of adriamycin plus vincristine. Combinations of adriamycin plus cyclophosphamide and 5-FU appear to be at least as good as the combinations which delete 5-FU. The remaining trials listed in the table are difficult to evaluate due to the small number of patients involved.

Randomized Trials Involving Adriamycin

There are at least 12 randomized trials involving adriamycin comparisons in patients not previously exposed to chemotherapy. The relative effectiveness of adriamycin compared to combination chemotherapy has been tested in the studies shown in table 12. The Southwest Cooperative Oncology Group data, while demonstrating nearly equivalent responses for adriamycin alone and two five-drug combinations, also showed that the duration of the response to adriamycin was inferior to that of the combinations. Similar results were obtained at the Mayo Clinic comparing adriamycin to three- and four-drug combinations. At the Albany Medical Center and Roswell Park Memorial Institute the response rate to adriamycin, although equivalent to adrenalectomy, appeared to be inferior to a three-drug combination. The duration of the adriamycin response in this latter trial was once again inferior in the early data. It is still too early to comment on the ECOG data.

Table 13 shows two trials from the Mayo Clinic with combinations containing adriamycin plus meth-

TABLE 13.—Comparative randomized trials utilizing ADR-MTX or ADR-MTX-VCR combinations*

Study group	Drug regimens†	No. of patients— Evaluated	Responding (%)	Mean duration of response (mos)
MAYO	CYT, FU, PRED	12	7 (58)	9+
	CYT, FU, PRED, VCR	10	6 (60)	7+
	ADR, MTX	24	10 (42)	8+
MAYO	CYT, FU, PRED	14	7 (50)	13+
	CYT, FU, PRED, CAL‡	11	5 (45)	14+
	ADR, MTX, VCR	23	12 (52)	7+

*Patients with prior chemotherapy were not eligible for these trials.
†All regimens have >13 days between drug courses.
‡CAL = calusterone.

TABLE 14.—Comparative randomized trials utilizing ADR combinations*

Study group	Drug regimens†	No. of patients— Evaluated	Responding (%)	Mean duration of response (mos)
MILAN	ADR, VCR (I)	19	10 (53)	3+
	ADR, VCR, PRED (I)	20	10 (50)	3+
MILAN	CYT, MTX, FU (I)	40	19 (48)	4+
	ADR, VCR (I)	40	19 (48)	4+
ECOG	CYT, MTX, FU (I)	31	20 (64)	5+
	ADR, VCR (I)	31	14 (45)	6+
NCI	CYT, MTX, FU (I)	32	18 (56)	10
	CYT, ADR, FU (I)	28	19 (68)	10+
ALGB‡	CYT, MTX, FU, VCR, PRED (C)	7	2 (28)	TE¶
	CYT, MTX, FU, VCR, PRED (I)	3	2 (67)	TE
	CYT, ADR, FU, VCR, PRED (I)	3	2 (67)	TE
MAYO, ALB, and BUF‡	CYT, FU, PRED (I)	TE	TE	TE
	CYT, FU, PRED, CAL§(I)			
	CYT, ADR (I)			
	CYT, ADR, FU (I)			
	CYT, FU, PRED (I) alternating with CYT, ADR (I)			

*Patients with prior chemotherapy were not eligible for these trials.
† C = weekly therapy; I = >13 days between courses.
‡Ongoing trial.
§CAL = calusterone.
¶ TE = too early to evaluate.

otrexate. In the first trial an adriamycin and methotrexate combination does not appear to be better and is perhaps inferior to the combination of cyclophosphamide, 5-FU, and prednisone with or without vincristine. However, the durations of the responses in the three regimens appear to be equivalent. The addition of vincristine to the methotrexate and adriamycin regimen was associated with a slightly higher response rate but no improvement in the remission duration in the early data.

An adriamycin and vincristine combination has been tested in Milan against an adriamycin, vincristine, and prednisone regimen and no differences in the response rates or durations were observed (table 14). Similarly, no significant difference was observed between an adriamycin and vincristine combination and a cyclophosphamide, methotrexate, and 5-FU combination (table 14). In a similar trial, the ECOG is showing a trend towards a higher response rate for the cyclophosphamide, methotrexate, and 5-FU combination (table 14). The cumulative response rate to cyclophosphamide, methotrexate, and 5-FU in these studies is 55%, and the cumulative response rate to adriamycin and vincristine in these trials is 46%. At the NCI we are observing a trend toward an advantage for cyclophosphamide, adriamycin, and 5-FU over cyclophosphamide, methotrexate, and 5-FU (table 14). It is too early to comment on the current study in the ALGB.

The remaining prospective comparative trial in table 14 is from the Mayo Clinic/Albany Medical Center/Roswell Park group. The data are too preliminary for comment.

DISCUSSION

The presentation of cumulative data from a variety of trials in which the definitions and patient data are not yet all available is fraught with interpretive difficulties. It is hoped that, despite these drawbacks, the preceding resumé will aid the investigator in selecting new and unique approaches to the use of adriamycin and at the same time provide some basis for the selection of the more appropriate therapeutic approaches for patients. It is unfortunate that there is still not enough meaningful data on toxicity, long-term response durations, and information concerning the value of pretherapy discriminants and their impact upon response rates and durations.

Despite these problems it appears that adriamycin is perhaps the most active single agent in the treatment of metastatic breast cancer, and for this reason it is being investigated in a wide variety of combinations. The activity of this drug is shown by its response rate of 43% in those patients without prior chemotherapy and 28% in those patients previously exposed to chemotherapy. This is better than the cumulative response rates obtained with some of the other more active single agents such as cyclophosphamide and methotrexate (1).

The data do seem to support the contention that the response rate to adriamycin as a single agent is nearly equivalent to that of many of the combination regimens; however, the duration of those responses appears to be shorter by a factor of 1½ to 3. For this reason alone the use of combinations would appear to be a more judicious path than the use of the single agent. Trials testing the use of intensive combinations following single-agent therapy with adriamycin may in the future obviate the need to go to combinations initially, but this type of data is not yet available. It would appear that combinations of adriamycin with alkylating agents produce superior response rates to the single agent alone. On the other hand, those combinations employing methotrexate or vincristine with adriamycin do not yet appear to have been as successful as the adriamycin-alkylating agent combinations.

The trials evaluating adriamycin in a variety of combinations, while appearing to provide superior response rates and response durations than adriamycin used alone, are still too preliminary to adequately evaluate the patient populations involved and the toxicities encountered. At the present time it would be somewhat difficult to believe that any one of these regimens is the superior regimen, and for that reason an open mind must be kept until the results of the ongoing trials are obtained.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
Calusterone: NSC-88536; CAS reg. No. 17021-26-0; testosterone, 7 β, 17-dimethyl-androst-4-en-3-one, 17β-hydroxy-7 β,17-dimethyl
CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan
Dibromodulcitol: NSC-104800; CAS reg. No. 10318-26-0; galactitol, 1,6-dibromo-1,6-dideoxy
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[p-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-
Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Adriamycin (NSC-123127) in the Treatment of Advanced Breast Cancer: Studies by the Southwest Oncology Group[1,2]

Barth Hoogstraten[3,4]

SUMMARY

The Southwest Oncology Group has studied the efficacy of adriamycin in the treatment of 315 patients with advanced breast cancer. Doses ranged from 25 to 75 mg/m² administered every 3 weeks. Drug side effects consisted mainly of gastrointestinal toxicity, leukopenia, and alopecia. Cardiomyopathy was noted when the total dose exceeded 500 mg/m². Response rates were 30% in 238 patients who had received prior chemotherapy with other agents and 40% in 77 patients who had not received prior chemotherapy. Eighty percent of the responses occurred with or before the fourth dose. The median duration of response was 4-5 months.

[Cancer Chemother Rep Part 3, vol 6:329-334, 1975]

During the past 4 years, the members of the Southwest Oncology Group (SWOG) have been involved in an extensive evaluation of the new anthracycline antibiotic, adriamycin, and it soon became apparent that this drug would play a significant role in the management of patients with breast cancer. In November 1970, the Group initiated a phase II study of adriamycin in a variety of human neoplasia, protocol study No. SWOG 442 (1), and the initial results were so encouraging that two studies with randomizations were started in January 1972: *(a)* study No. SWOG 449, for patients who had received an adequate trial with prior chemotherapy (2), and *(b)* study No. SWOG 450, for patients who had not received prior chemotherapy (3). All of these patients had metastatic breast cancer. In addition, the SWOG activated a dose-response study in April 1972 (study No. SWOG 7206), for patients with all types of metastatic cancer (4). These four studies have been closed and this report discusses the results derived from the evaluation of a total of 315 breast cancer patients. In addition, an outline is given of our new adriamycin combination study.

MATERIALS AND METHODS

To be eligible for these studies, the patients had to have histologic proof of disseminated breast carcinoma, they must not have received chemotherapy for at least 3 weeks prior to the study, and the nadirs of the white blood cell (wbc) and platelet counts resulting from any prior therapy must have passed. Patients who had received prior therapy with either adriamycin or daunorubicin[5] were not eligible. When hormone therapy had been used or terminated, such an event must have happened at least 6 weeks prior to entering the studies, and progressive disease had to be present. All patients signed an informed-consent form in keeping with institutional policies.

Patients with a history of cardiac disease were not included and in order to further avoid cardiac toxicity of adriamycin, the total dose administered was limited to 550 mg/m² in all but the first study. Patients with prior chemotherapy were separated into *(a)* a good-risk group whose patients had adequate bone marrow reserve as determined by an initial wbc greater than 5000 cells/mm³ and a platelet count greater than 150,000 platelets/mm³, plus good tolerance to the prior therapy, and *(b)* a poor-risk group whose patients had inadequate bone marrow reserve. The poor-risk group was defined as patients who had experienced poor tolerance to prior chemotherapy, or had had extensive prior radiotherapy, or were over 70 years of age. In addition, the patients in the poor-risk group did not have to meet the previously mentioned minimum wbc and platelet count values.

In study No. SWOG 442, some good-risk patients initially received courses of 25 mg/m² daily for 3 days which were repeated every 3 weeks, and the poor-risk patients received 20 mg/m² given on the same schedule. However, these doses were soon changed to 75 and 60 mg/m², respectively, given by single intravenous (iv) injections which were repeated every 3 weeks (table 1). The same single doses were used in study No. SWOG 449, but only 60 mg/m² was

[1]Supported by Public Health Service grant CA-12644 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[2]Adriamycin: CAS reg. No. 23214-92-8. Supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

[3]Department of Internal Medicine, Division of Clinical Oncology, The University of Kansas Medical Center, College of Health Sciences and Hospital, Kansas City.

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[5]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

TABLE 1.—SWOG studies of adriamycin in the treatment of breast cancer

SWOG study No.	Dose* (mg/m^2)	Patient classification†	No. of patients	Response (%)	Duration of response (mos)
442	75	PT+GR	50	32	4
	60	PT+PR			
449	75	PT+GR	45	33	7
	60	PT+PR			
450	60	NPT	77	40	5
7206	75	PT+GR	35	29	
	60	PT+GR	24	38	
	45	PT+GR	24	42	3
	50	PT+PR	32	19	
	25	PT+PR	28	15	
Totals		NPT	77	40	5
		PT	238	30	4

*All doses repeated q3wks.
†PT = prior chemotherapy; GR = good-risk patient; PR = poor-risk patient; NPT = no prior chemotherapy.

given in study No. SWOG 450, which did not have the good- and poor-risk division. In study No. SWOG 7206 we used three doses for the good-risk patients (75, 60, and 45 mg/m^2) and two doses for the poor-risk group (50 and 25 mg/m^2). In each of these studies the subsequent doses were reduced if myelosuppression occurred.

Patients were followed up with weekly blood cell counts, and with ECGs, liver and renal function tests, and tumor measurements at 3-week intervals, although these requirements varied slightly in the different protocols.

In all of our studies a complete response was defined as the disappearance of all clinical evidence of tumor for a minimum of 4 weeks. In patients with both soft tissue and bone metastases this does not necessarily mean complete normalization of an involved skeleton but there must be improvement on X-ray films. A partial response was defined as at least a 50% or greater decrease in the sum of the products of the diameters of all measured lesions for a minimum of 4 weeks with no simultaneous increase in the size of any lesions nor the appearance of new lesions. Since so few complete responses occurred, the term *response* will include the complete and partial responses unless specifically stated differently.

TOXICITY

The degree of leukopenia resulting from drug therapy in the SWOG is defined in table 2. Platelet depression is also included in this table, but it should immediately be stated that adriamycin has little or no effect on the platelet count. Significant leukopenia is the major hematologic side effect of adriamycin and details are shown for two studies in table 3. Higher toxicity was apparent in the patients with prior chemotherapy, and in these patients the mean degree of leukopenia appeared to follow a dose-toxicity curve. In patients who had received no prior chemotherapy, this relationship is not apparent. Only the patients receiving the 75-mg/m^2 dose stand out. This may, at least in part, be a reflection of the small number (11 patients) treated with the 60-mg/m^2 dose in study No. SWOG 7206, because in study No. SWOG 450, we observed in 77 patients the much more accurate mean of 1.64. It should be remembered that the patients from study No. SWOG 7206 in table 3 include many patients with carcinomas other than breast cancer and that the breast cancer patients usually had received more radiation therapy than other patients.

TABLE 2.—Definitions of degrees of hematologic toxicity

Degree	Wbc ($cells/mm^3$)	Platelets ($platelets/mm^3$)
0	≥4000	≥100,000
1	3000-3999	75,000-99,999
2	2000-2999	50,000-74,999
3	1000-1999	25,000-49,999
4	<1000	<25,000

TABLE 3.—Leukopenia resulting from adriamycin therapy

SWOG study No.	Dose (mg/m²)	Total No. of patients	No. of patients experiencing degree of leukopenia‡					Mean
			0	1	2	3	4	
450*	60	77	18	18	17	22	2	1.64
7206*	75	75	21	14	15	17	8	1.70
	60	11	7	3			1	0.64
	50	19	8	5	5	1		0.95
	45	52	31	10	4	5	2	0.79
	25	17	11	1	5			0.65
7206†	75	105	14	15	25	37	14	2.21
	60	32	8	2	6	8	8	2.19
	50	83	21	12	16	21	13	1.92
	45	78	26	10	20	18	4	1.54
	25	69	24	15	12	8	10	1.50

*No prior chemotherapy.
†Prior chemotherapy.
‡See table 2 for definitions of degrees.

The degree of myelosuppression that occurred in patients with liver involvement was measured with great accuracy in study No. SWOG 450, and there was no difference at all between the 1.68 mean degree of leukopenia that occurred in 53 patients without liver metastases and the 1.64 mean in 24 patients with such metastases.

Alopecia develops in all patients receiving adriamycin and most patients become completely bald. Hair growth sometimes starts again while patients are still receiving the drug. Nausea and vomiting were reported in approximately two thirds of the patients by the investigators but were definitely severe in only 10%-15%. These toxic effects resulted in dose modifications in some of the patients and six patients refused further treatment although three of them had achieved a partial response. Stomatitis occurred in a small number of patients, as did skin darkening.

Of special interest is the cardiac toxicity. The SWOG has now used adriamycin in over 1500 patients and Gottlieb et al reported on the incidence of toxicity in the first 760 cases (5). In 719 patients, at a total dose of less than 550 mg/m², only one case of cardiomyopathy was observed, whereas in the 41 patients who received more than 550 mg/m² there were 11 cases including eight deaths. We now routinely keep the total dose below 500 mg/m². At that dose, only two cases of congestive heart failure were observed in the 77 patients in study No. SWOG 450 and this effect was corrected with digitalization in both patients.

RESULTS

The results of our four studies involving adriamycin are shown in table 1. The majority of the patients received either 75 or 60 mg/m² at 3-week intervals and all patients with prior chemotherapy were stratified into the good-risk and poor-risk categories. Only study No. SWOG 450 was designed to use adriamycin in patients without prior chemotherapy. Such patients were randomized to receive either a weekly five-drug combination, an intermittent 5-day course of the same five drugs repeated every 4 weeks, or adriamycin every 3 weeks.

Noticeable is the consistent percentage of patients responding to adriamycin in the good- and poor-risk groups that received prior chemotherapy: 32% (SWOG 442), 33% (SWOG 449), and 32% (SWOG 7206), as long as the doses were 75 or 60 mg/m². It can further be observed that in this last study the prior-chemotherapy good-risk group did just as well with the 45-mg/m² dose and that those patients had only 19% and 15% response with 50 and 25 mg/m², respectively. In all three studies for the patients with prior chemotherapy, the ratio of response for the good-risk and the poor-risk groups was approximately 2:1, about 40% for good-risk and 20% for poor-risk patients.

Of interest is the 40% response rate for patients who had received no prior chemotherapy. Adriamycin appears to do slightly better in these patients. The median time intervals from the start of chemotherapy to response were 2 months in study No. SWOG 449 in patients with prior chemotherapy and 45 days in study No. SWOG 450 (fig 1). In this last study, 80% of the responding patients responded at 80 days, which is after the fourth dose.

The duration of the responses was disappointingly short: 4, 7, 5, and 3 months respectively, in the four studies (table 1). Overall there is again no difference between the no-prior-chemotherapy and the prior-chemotherapy patients. The duration ranged between 1 and 16+ months.

The response by metastatic site of involvement can only be determined in study Nos. SWOG 449 and SWOG 450 which were started simultaneously (table 4). Responses were seen in all patients except those with central nervous system metastases. The slightly higher response in study No. SWOG 450 is also reflected in the overall response (table 1). The lowest

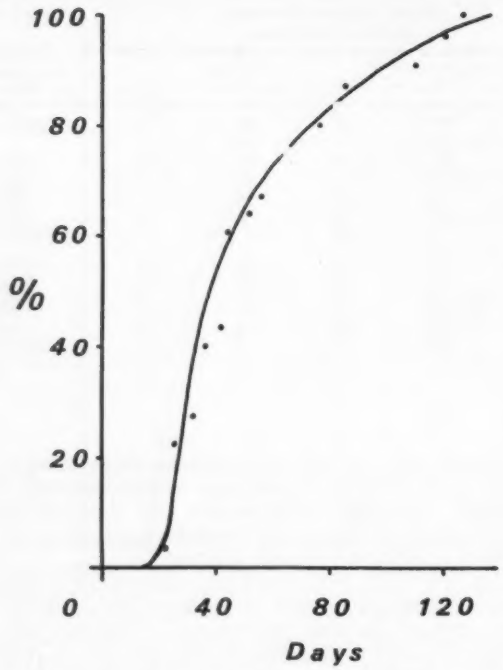

FIGURE 1.—Median time intervals from the start of adriamycin therapy to response.

objective response rate was in patients with skeletal metastases, but about one half of the patients had impressive relief from bone pain as well. The response seen in some patients with liver metastases was satisfying, an example of which is shown in figures 2*A* and 2*B*

Study No. SWOG 450 was analyzed to determine the possible influence of age, menopausal status, and duration of disease on the results of adriamycin therapy. Table 5 shows that patients at an age of 55 or less at the time of their diagnosis had fewer remissions than their older counterparts. The same can be seen in the ages at the start of study No. SWOG 450. One third (11 of 33) of the patients who were premenopausal at the time of their diagnosis responded as compared to 43% (19 of 44) of the postmenopausal women. Remission occurred less frequently when the interval between the date of diagnosis and the start of the study was between 12 and 23 months (table 6).

Only seven of 59 patients with an interval of 12 months or longer had a simple mastectomy, but seven of the 18 patients in the shortest-interval group had either a simple mastectomy (4) or only a biopsy (3), and five of these patients responded. This limited surgical treatment was a palliative measure in these patients with aggressive carcinoma. These patients also had the shortest responses.

DISCUSSION

There is little doubt that adriamycin is a new drug with considerable activity in breast cancer and that

FIGURE 2.—*A.* Liver scan on 6/13/73 showing large metastases. *B.* Liver scan on 1/7/74 after adriamycin therapy, 470 mg/m^2.

TABLE 4.—Response by metastatic site

Metastatic site	No. of patients with—* Prior chemotherapy (Study No. 449)	No prior chemotherapy (Study No. 450)
Skin, soft tissue	11/24 (46)	17/32 (53)
Lungs	5/17 (30)	11/31 (36)
Liver	2/10 (20)	5/18 (28)
Bones	4/21 (19)	8/43 (19)

*No. of patients responding/total No. of patients in that category. Numbers in parentheses = % of patients responding.

TABLE 5.—Age of responding patients at time of diagnosis and at start of study No. SWOG 450

Age (yrs)—	No. of responders/ No. of patients treated
At time of diagnosis	
≤45	9/26 (35%)
46-55	11/31 (35%)
>55	11/20 (55%)
At start of study No. SWOG 450	
≤45	3/12 (25%)
46-55	12/34 (35%)
>55	16/31 (51%)

it is comparable to if not better than any of the other available standard agents. Only cyclophosphamide and methotrexate have, over the years, proved to be effective in about one third of the patients in series which are large enough that we can accept the results with confidence (6).[6]

The most commonly used doses in our studies were 75 and 60 mg/m^2 once every 21 days, but recently we have also seen good results with lower doses which lead to less toxicity. The side effects of adriamycin cannot be minimized and although the cardiac toxicity has been almost avoided with the use of a total dose of less than 500 mg/m^2, one should not overlook the marked gastrointestinal disturbances, the myelosuppressive effects, and the severe alopecia, which is objectionable in some patients. It must be remembered that the late sequelae are entirely unknown. In patients with skeletal metastases the considerable relief in bone pain can outweigh the toxic effects.

The 40% response rate in patients who had no prior chemotherapy and the efficacy of adriamycin in treated patients who had failed to respond or who had experienced a relapse from combination chemotherapy with standard agents are indeed impressive. But the responses lasted a disappointingly short period, with some relapses occurring while the patients were still receiving the drug. When one combines this observation with our experience that 80% of the responding patients had achieved their remission after four doses, then one comes to the conclusion that adriamycin should probably be given in a different schedule. The SWOG is building on the results obtained in its four studies and is currently investigating three combinations of adriamycin and standard agents (table 7). Taking full advantage of the response rate with four doses of adriamycin, and of the median duration of response of 4 months, we are giving the drug in courses of four injections, each followed by an interval of 3 months. At the same time, each dose has been reduced to 40 mg/m^2, resulting in less gastrointestinal toxicity and thus making the therapy more acceptable to patients.

We anticipate that adriamycin will make a significant contribution to the management of breast cancer, recognizing that much still has to be learned about its dosimetry, its dose schedule, and its role in combination chemotherapy.

[6]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

TABLE 6.—Influence on response of time interval between diagnosis and study No. SWOG 450*

Time interval (mos)	No. of patients responding	No. of patients showing response and improvement
12	9/18 (50)	10/18 (56)
12-23	4/17 (24)	4/17 (24)
24-59	11/25 (44)	12/25 (48)
60	7/17 (41)	12/17 (71)

*No. of patients responding/total No. of patients. Numbers in parentheses = % of patients responding.

TABLE 7.—Current SWOG protocol for combination chemotherapy for breast cancer

Combination No.	Drug	Dose (mg/m^2)	First cycle (days)								Drug given during 12-wk interval*	Second cycle (days)				
			1	8	22	29	43	50	64	71		168	175	189	196	etc
I	Adriamycin +	40	×		×		×		×			×		×		
	5-FU†	500	×	×	×	×	×	×	×	×	5-FU	×	×	×	×	
II	Adriamycin +	40	×		×		×		×			×		×		
	5-FU +	400	×	×	×	×	×	×	×	×	5-FU +	×	×	×	×	
	Cyclophosphamide	400	×		×		×		×		Cyclophosphamide	×		×		
III	Adriamycin +	40	×		×		×		×			×		×		
	5-FU +	300	×	×	×	×	×	×	×	×	5-FU +	×	×	×	×	
	Cyclophosphamide +	300	×		×		×		×		Cyclophosphamide +	×		×		
	Methotrexate	10	×		×		×		×		Methotrexate	×		×		

*During the 12-wk interval all drugs continue except adriamycin.
†5-FU: NSC-19893; CAS reg. No. 51-21-8; 5-fluorouracil.

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Evaluation of Combination Therapy With Adriamycin (NSC-123127) and Methotrexate (NSC-740) in Patients With Disseminated Breast Cancer[1,2]

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SUMMARY

Forty-six patients with disseminated breast cancer were evaluated in a prospective randomized clinical trial contrasting the combination of adriamycin and methotrexate with the combination of 5-fluorouracil (5-FU), cyclophosphamide, and prednisone with or without vincristine. Nine of 24 patients responded to adriamycin and methotrexate as compared to 13 of 22 patients receiving the other combinations (seven of 12 without vincristine and six of ten with vincristine). Toxicity from the combination of methotrexate and adriamycin was tolerable, although dose reductions were necessary in the majority of patients because of excessive myelosuppression. Gastrointestinal toxic effects and alopecia were also more common and more severe with the two-drug combination compared to the combination of 5-FU, cyclophosphamide, and prednisone. Secondary responses utilizing the adriamycin/methotrexate regimen seemed less effective than those resulting from primary therapy, as was also true of the combination of 5-FU, cyclophosphamide, and prednisone. Perhaps the major advantage of this particular adriamycin combination as opposed to adriamycin used as a single agent would be in the duration of regression in patients with objective tumor response. Since smaller individual adriamycin doses were utilized, this drug could be given for longer periods of time before reaching dose-limiting cumulative toxicity.

[Cancer Chemother Rep Part 3, vol 6:335-338, 1975]

Adriamycin was found to have a 50% response rate in patients with advanced breast cancer in a previous phase II evaluation at this institution (1). Regression rates associated with the utilization of adriamycin in patients with-advanced breast cancer have varied somewhat in other reports (2,3) but adriamycin could be stated to be among the most active single agents currently available for this disease.

Methotrexate, used as a single agent, has also shown definite activity in patients with disseminated breast cancer (4). It has been included in many multiple-drug programs because of its primary antitumor effect and its ability to combine well in these programs.

Since our experience utilizing one multiple-drug treatment regimen of 5-fluorouracil (5-FU), cyclophosphamide, and prednisone[6] has consistently yielded a 50% or better response rate in patients with disseminated breast cancer, it seemed desirable to establish another multiple-drug regimen utilizing other agents which would not show cross resistance to the former program. Therefore, this study was designed to assess the antitumor effect of adriamycin and methotrexate, a combination of agents not utilized in our standard multiple-drug program. A crossover design upon primary drug failure was incorporated into the study design to test for cross resistance.

MATERIALS AND METHODS

Forty-six patients with disseminated breast cancer were consecutive candidates for cytotoxic chemotherapy and were included in this study after criteria for admission were fulfilled. All had metastatic,

[1]Supported in part by contract N01-CM-12185 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[2]Adriamycin: CAS reg. No. 23214-92-8; ADR.

Methotrexate: CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-; MTX.

All drugs used in this study were supplied by Cancer Therapy Evaluation, DCT, NCI.

[3]Division of Medical Oncology, Mayo Clinic, Rochester, Minn.

[4]The authors wish to gratefully acknowledge Drs. Douglass Tormey, Stephen Carter, and Paul Carbone, DCT, NCI, for assistance and advice, and Dr. William F. Taylor and Mrs. Helen Golenzer, Department of Statistics, Mayo Clinic, for statistical support and randomization techniques.

[5]*Reprint requests to:* David L. Ahmann, MD, Division of Medical Oncology, Mayo Clinic, Rochester, Minn 55901.

[6]5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU.

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan; CTX.

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1, 4-diene-3,11,20-trione, 17,21-dihydroxy-; PRED.

recurrent, or inoperable primary breast cancer for which no better treatment was available. Breast cancer had been confirmed histopathologically in every patient and there were no second primaries present in this group that might have made the origin of the metastatic lesions questionable. Metastatic lesions were quantitatively assessable on serial measurements.

All of the patients were postmenopausal and had shown progression of metastatic disease if a previous hormonal program had been employed. Many of the patients had received hormonal therapy previously but none of the patients had received cytotoxic chemotherapy prior to entry in this study. None of the indicator lesions chosen for serial measurement had been treated with radiation and no patient had received radiation therapy to an extent that would have compromised bone marrow reserve.

Prior to entering this study, the investigational aspects, the possible toxicity of the agents utilized, and the limitations of the chemotherapeutic programs were discussed thoroughly with every patient.

The initial evaluation was as previously outlined (5) and also included an electrocardiogram. None of the patients had abnormal renal function (serum creatinine > 1.5 mg/100 ml) or hyperbilirubinemia (serum bilirubin > 2 mg/100 ml). The white blood cell count (wbc) exceeded 4100 cells/mm^3 and the platelet count exceeded 130,000 platelets/mm^3 in all patients. No patients were entered in this study with active cardiac disease.

Before random assignment to the treatment programs, the patients were classified according to free interval, dominant disease, and interval since last menstruation (table 1) to achieve study balance. The multiple-drug program of 5-FU, cyclophosphamide, and prednisone with or without vincristine[7] served as a control for the methotrexate/adriamycin evaluation.

Twenty-four patients were randomly allocated to the combination of adriamycin and methotrexate as primary therapy and 22 patients received the combination program of 5-FU, cyclophosphamide, and prednisone with or without vincristine.

Adriamycin was administered at an initial dose of 60 mg/m^2 as a single intravenous (iv) infusion on Day 1, while methotrexate was given at a dose of 15 mg/m^2 as an iv infusion on Days 1 and 4. For the most part methotrexate was given 4 hours prior to the dose of adriamycin on Day 1. Subsequent courses, when administered, required a reduction of the adriamycin and methotrexate doses,usually because of excessive myelosuppression. A cumulative dose of adriamycin of 550 mg/m^2 was not exceeded, but when approached, crossover to the other treatment program was accomplished.

The group receiving 5-FU, cyclophosphamide, and prednisone received 5-FU at a dose of 300 mg/m^2 and cyclophosphamide at a dose of 150 mg/m^2 on each of 5 successive days by rapid iv infusion. Prednisone was given orally at a dose of 30 mg/day for the first 2 weeks, 20 mg/day for the third week, and 10 mg/day thereafter as a maintenance dose without regard to body weight. By random assignment ten patients were given vincristine in addition to the multiple-drug program of 5-FU, cyclophosphamide, and prednisone at a dose of 1.4 mg/m^2 on Days 1 and 5 by iv

[7]Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); VCR.

TABLE 1.—Distribution of patients and response by discriminants: adriamycin/methotrexate vs multiple-drug programs*

Discriminant	No. of responses/No. of patients receiving— Adriamycin/ methotrexate	5-FU/ cyclophosphamide/ prednisone	5-FU/ cyclophosphamide/ prednisone/ vincristine
Dominant disease			
Visceral	7/18	4/8	2/5
Osseous	1/4	1/1	2/3
Soft tissue	1/2	2/3	2/2
Postmenopausal status (yrs)			
<1	1/4	2/2	2/3
1-5	5/8	1/2	2/3
5-10	1/6	3/4	1/2
>10	2/6	1/4	1/2
Free interval (yrs)			
<1	3/8	1/3	2/2
1-5	4/9	3/6	3/6
>5	2/7	3/3	1/2
Total	9/24	7/12	6/10

*Primary response only.

infusion (not to exceed 2 mg). The doses of 5-FU, cyclophosphamide, and prednisone were rarely reduced; however, vincristine was reduced in seven of ten patients in subsequent courses because of excessive neurologic toxic effects.

Both groups were reevaluated 4 weeks after the start of treatment at which time the physical examination, measurement of indicator lesions, and pretreatment assessment were repeated. Wbc and platelet counts were obtained periodically for 3 weeks after therapy. If an objective regression had occurred, treatment with the same program was repeated; if the first program had failed, a crossover assignment to the other treatment program was made. When the disease remained objectively and subjectively stable, the same program was continued until there was objective evidence of progressive disease. Patients who failed both chemotherapeutic programs were removed from the study but followup was continued.

In patients with visceral and soft tissue metastases, an objective regression was defined as a reduction in the malignant lesion(s) by at least 50% of the product of the greatest perpendicular diameters. An osseous lesion must have remained stable or have shown healing. For metastatic liver disease, a reduction of 30% in the sum of the linear measurements of the liver below each costal margin at the midclavicular line and the xiphoid must have occurred. Metastatic disease was considered to be progressive if any new lesion occurred or if any metastatic lesion increased in size by greater than 25% after therapy.

RESULTS

Table 2 indicates the frequency of regressions obtained within each of the study programs. Appreciable antitumor response was apparent utilizing methotrexate and adriamycin, with nine of 24 patients showing objective responses. The median and mean duration of responses thus far is 34 weeks. In three patients, regressions are being maintained although the patients have crossed over to the alternate treatment program because the cumulative dose of 550 mg/m^2 of adriamycin was reached. Regression durations were measured from the onset of treatment until the cumulative dose of 550 mg/m^2 was reached. Therefore, durations of regressions are limited.

TABLE 2.—Response rates: adriamycin/methotrexate vs 5-FU/cyclophosphamide/prednisone with or without vincristine

	No. of responses/ No. of patients treated
Primary treatment	
Adriamycin/methotrexate	9/24
5-FU/cyclophosphamide/prednisone	7/12
5-FU/cyclophosphamide/prednisone/ vincristine	6/10
Secondary treatment	
Adriamycin/methotrexate	2/10
5-FU/cyclophosphamide/prednisone	5/13*
5-FU/cyclophosphamide/prednisone/ vincristine	4/9†

*One maintained adriamycin/methotrexate response.
†Two maintained adriamycin/methotrexate responses.

Objective responses to 5-FU, cyclophosphamide, and prednisone were seen in seven of 12 patients with a median duration of 34 weeks (mean = 36 weeks), while three patients remain in regression at 46, 52, and 52 weeks at the time of this report. The combination of 5-FU, cyclophosphamide, and prednisone with vincristine was effective in six of ten patients with a median duration of 35 weeks (mean = 34 weeks), while one patient remains in regression at 57 weeks. Both the response rate and duration of responses seem quite comparable between the regimen of adriamycin plus methotrexate and the multiple-drug program of 5-FU, cyclophosphamide, and prednisone with or without vincristine.

Secondary therapy seemed less effective with all of the treatment programs. Thus far, two of ten patients have responded to the combination of adriamycin and methotrexate (the durations currently are 16 and 32 weeks), while five of 13 patients and four of nine patients have responded to 5-FU, cyclophosphamide, and prednisone with or without vincristine respectively. Three of these nine patients, however, have responses that were originally obtained utilizing the combination of adriamycin and methotrexate and are being maintained with the combination programs. The mean duration of secondary responses to 5-FU, cyclophosphamide, and prednisone with or without vincristine is currently in the range of 25 weeks.

The toxicity encountered utilizing these combination programs is displayed in tables 3 and 4.

The combination of 5-FU, cyclophosphamide, and prednisone with or without vincristine produced much the same toxicity as was previously reported (5) and is associated with only moderately severe myelosuppression of the platelet-sparing type. As experienced previously, the myelosuppression seemed more profound with the addition of vincristine. Neurologic toxic effects were confined to the vincristine treatment group.

The initial dose utilized in the combination of adriamycin and methotrexate proved excessive since 11 of 16 patients receiving this combination required dose reduction during a subsequent course, averaging 20% for adriamycin and 30% for methotrexate, usually because of excessive myelosuppression. Stomatitis occurred in 50% of the patients receiving the adriamycin/methotrexate combination but in only two patients was it severe enough to markedly alter alimentation. Myelosuppression was the most prominent toxic effect from adriamycin and methotrexate and the mean nadir of leukocyte depression was below that seen with the other multiple-drug treatment programs concurrently evaluated. Cardiac toxic effects were not encountered in the adriamycin treatment group in this clinical trial.

TABLE 3.—Toxicity encountered: adriamycin/methotrexate vs 5-FU/cyclophosphamide/prednisone with or without vincristine*

	Toxic effects†					
	Nausea	Vomiting	Diarrhea	Stomatitis	Neurologic	Alopecia
Adriamycin/methotrexate	29/34	25/34	8/34	17/34	0/34	34/34
5-FU/cyclophosphamide/prednisone	20/25	12/25	1/25	2/25	0/25	16/25
5-FU/cyclophosphamide/prednisone/vincristine	12/19	5/19	5/19	2/19	15/19	16/19

*Primary and secondary treatment included.
†No. of patients with toxic effects/total No. of trials.

TABLE 4.—Hematologic toxicity encountered: adriamycin/methotrexate/5-FU/cyclophosphamide/prednisone with or without vincristine*

Treatment	No. of patients with toxicity/ No. of patients treated	
	Leukocyte count <3000 cells/mm³	Platelet count <100,000 platelets/mm³
Adriamycin/methotrexate	30/34†	12/34
5-FU/cyclophosphamide/prednisone	11/25‡	1/25
5-FU/cyclophosphamide/prednisone/vincristine	14/19§	1/19

*Primary and secondary treatments included.
†Mean nadir = 1.6×10^3 cells/mm³; duration = 8.3 days.
‡Mean nadir = 2.3×10^3 cells/mm³; duration = 6.6 days.
§ Mean nadir = 2.1×10^3 cells/mm³; duration = 7 days.

DISCUSSION

The response rate of the combination of adriamycin with methotrexate utilized in this treatment regimen was in the same statistical range as the multiple-drug program previously experienced (1) and employed as a control arm in this study. It seems that the addition of methotrexate to adriamycin failed to increase the response rate associated with this group of patients when compared to a similar group of patients who had received adriamycin alone as primary therapy (1). Because of the concomitant reduction in adriamycin doses, however, adriamycin, when effective, may be continued for a longer period of time until the cumulative dose of 550 mg/m² is reached.

The duration of response with adriamycin and methotrexate thus far seems quite comparable to that observed during previous studies utilizing adriamycin alone. The duration of response is also quite comparable at this time with the duration of responses associated with the multiple-drug programs utilizing 5-FU, cyclophosphamide, and prednisone with or without vincristine.

Clinical trials should continue utilizing various combinations of drugs capitalizing on the primary antitumor effect of adriamycin. We must be cognizant of the importance of reducing the adriamycin dosage as much as possible without sacrificing primary response rates. Particular emphasis should also be placed on the importance of improving the duration of responses in patients treated for disseminated breast cancer, probably by designed fixed crossover treatment programs.

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Controlled Evaluation of the Combination of Adriamycin (NSC-123127), Vincristine (NSC-67574), and Methotrexate (NSC-740) in Patients With Disseminated Breast Cancer[1,2]

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SUMMARY

Forty-nine patients with disseminated breast cancer were evaluated in a prospective randomized trial which contrasted the combination of vincristine, methotrexate, and adriamycin with the combination of 5-fluorouracil, cyclophosphamide, and prednisone (with or without the addition of calusterone). Eleven of 23 patients responded to the adriamycin combination as compared to 14 of 26 patients receiving the other treatments (six of 12 with calusterone, eight of 14 without). There were no significant differences in the response rates. Toxic effects, particularly neurologic impairment, myalgias, anorexia, and hematologic depression, were either more severe or more frequent in the adriamycin, vincristine, and methotrexate combination; no cardiac toxicity was seen. The response rates for the adriamycin combination used as secondary treatment were poor (none of seven) compared to responses for the other combinations (three of eight).

[Cancer Chemother Rep Part 3, vol 6:339-342, 1975]

In an extended phase II clinical evaluation of adriamycin, O'Bryan et al (1) reported a 32% response rate in previously treated women with disseminated breast cancer. We have reported a 50% (ten of 20 patients) response rate for adriamycin in previously untreated women with disseminated breast cancer (2). In an attempt to increase the percentage of patients responding, we tested the combination of adriamycin plus methotrexate and obtained a 39% (nine of 23 patients) response rate (3). Although this response rate was not superior to that achieved with adriamycin used alone, it was felt that the duration of response and overall rate of survival might have been improved (although the numbers were not large enough for statistical significance).

Both vincristine and methotrexate have activity in breast cancer with cumulative response rates of 20% and 34% respectively (4). Vincristine has been shown experimentally to increase the antitumor activity of methotrexate (5) and to be synergistic with adriamycin (6). Brambilla et al (7) have reported an improvement in response rate for the combination of adriamycin plus vincristine over adriamycin used alone. The alternative treatment programs employed in this randomized study utilized a combination of 5-fluorouracil, cyclophosphamide, and prednisone with or without the addition of calusterone. This regimen (without calusterone) has been previously reported by us and has been consistently associated with a 50% response rate in similar groups of patients (8). Calusterone, a new synthetic androgen, has been reported to produce a 28% response rate in postmenopausal women (9).

In this study we report on the efficacy of the combination of adriamycin, vincristine, and methotrexate.

MATERIALS AND METHODS

The 50 patients entered into our study had known metastatic breast cancer which was quantitatable and lent itself to serial measurement. One patient receiving vincristine, methotrexate, and adriamycin (schedule A) was removed from the study (with tumor response) after it was learned that she was receiving vitamin B_{17} (Laetrile). All patients were postmenopausal by virtue of natural menopause or oophorectomy and had progressive disease. Many patients had received prior hormonal therapy (except calusterone) but their tumors had progressed before they entered this study. No patient had had

[1]Supported in part by contract N01-CM-12185 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[2]All compounds used in this study were supplied by Cancer Therapy Evaluation, DCT, NCI. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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TABLE 1.—Distribution of patients and responses by discriminants*

Discriminant	Treatment schedule† A (ADR + VCR + MTX)	B_1 (5-FU + CTX + PRED)	B_2 (5-FU + CTX + PRED + CAL)	Totals
Dominant disease				
Visceral (liver, lung, and abdominal masses)	8/16	8/12	4/6	20/34
Osseous	0/2	—	1/3	1/5
Soft tissue (local [skin] recurrence and lymph nodes)	3/5	0/2	1/3	4/10
Totals	11/23	8/14	6/12	25/49
Menstrual category (yrs postmenopausal)				
<1	2/5	0/1	1/1	3/7
1-5	3/4	3/5	1/3	7/12
5-10	3/6	1/2	1/3	5/11
>10	3/8	4/6	3/5	10/19
Totals	11/23	8/14	6/12	25/49
Disease-free interval (yrs)‡				
<1	4/8	1/5	1/5	6/18
≥1	7/15	7/9	5/7	19/31
Totals	11/23	8/14	6/12	25/49

*No. of responses/No. of patients treated.
†ADR = adriamycin; VCR = vincristine; MTX = methotrexate; 5-FU = 5-fluorouracil; CTX = cyclophosphamide; PRED = prednisone; and CAL = calusterone.
‡Time from discovery and/or treatment of primary breast cancer to appearance of metastatic disease.

prior chemotherapy or prior or concomitant radiation therapy of the indicator lesion. All patients had adequate marrow function, ie, a white blood cell (wbc) count of ≥ 4100 cells/mm^3 and a platelet count of ≥ 130,000 platelets/mm^3 (institutional normals). All patients were informed of the nature of the study, its goals, its toxicity, etc, and had signed an informed consent.

The pretreatment evaluation has been previously reported (8) and is fairly standard for a phase II study. Electrocardiograms were obtained before a patient entered the study and before each dose of adriamycin. No patient had abnormal renal function (ie, a serum creatinine > 1.5 mg/100 ml) nor a serum bilirubin > 2 mg/100 ml. There was no evidence of active cardiac disease in any of these patients.

Before randomization to a treatment schedule, patients were stratified by site of dominant disease (visceral, > osseous, > soft tissue), menstrual category, and disease-free interval as shown in table 1.

Patients were assigned at random in a ratio of 2:1:1 to one of three treatment programs. Twenty-four patients received treatment schedule A: vincristine given at a dose of 1.4 mg/m^2 (2.0 mg maximum) on Days 1 and 5 followed by methotrexate, at a dose of 10 mg/m^2 6 hours later also on Days 1 and 5. On Day 2 (18-24 hours after the first dose of methotrexate), 40 mg/m^2 of adriamycin was given. Fourteen patients received treatment schedule B_1: 5-fluorouracil given at a dose of 300 mg/m^2/day and cyclophosphamide, 150 mg/m^2/day on each of 5 consecutive days. Prednisone was given orally at a dose of 30 mg/day for 2 weeks, 20 mg/day for 1 week followed by a daily maintenance dose of 10 mg/day. Twelve patients received treatment schedule B_2: 5-fluorouracil, cyclophosphamide, and prednisone administered identically to schedule B_1 plus 200 mg/day of calusterone given orally. Adriamycin, vincristine, methotrexate, 5-fluorouracil, and cyclophosphamide were all administered by direct intravenous infusion. All treatment schedules were repeated every 5 weeks after Day 1.

At each reevaluation, the pretreatment studies were repeated. If objective regression or stability of

disease had occurred, patients were re-treated with the same program. If the first treatment failed, patients on schedule A were randomized between schedules B_1 and B_2. All patients on schedules B_1 and B_2 received schedule A (providing eligibility criteria were met). Patients who failed two chemotherapeutic programs were removed from the study. Patients who were in remission status or had stability of disease while receiving schedule A and reached our maximum cumulative adriamycin dose of 540 mg/m^2 were randomized between schedules B_1 and B_2.

An objective regression was defined as a $\geq$ 50% reduction in the product of the two longest perpendicular diameters of a measurable lesion with no new or progressing lesions. If osseous disease was present, it must have improved or remained stable. In the event of hepatic metastases, a reduction by $\geq$ 30% of the sum of the linear measurements below each costal margin at the midclavicular line and xiphoid must have occurred. Progressive disease was defined as the appearance of any new lesion or an increase of > 25% in any preexisting lesion.

RESULTS

Table 1 indicates by discriminants the objective regressions observed with the three treatment schedules and table 2 indicates the overall response rate for each of these treatment programs.

TABLE 2.—Response to three treatment schedules*

Schedule†	Treatment	
	Primary	Secondary
A	11/23	0/7
B_1	8/14	1/3
B_2	6/12	2/5

*No. of responses/No. of patients treated.
†See table 1 for explanation of drug schedules.

Eleven of 23 patients responded to the combination of vincristine, methotrexate, and adriamycin (schedule A) used as initial therapy; eight of these patients had visceral disease. The median length of regression was 32 weeks (range, 7-52+ weeks). Nine patients have been in remission status from 25 to 52+ weeks. No patient responded to schedule A when it was used as secondary therapy.

Of 14 patients treated with the combination of 5-fluorouracil, cyclophosphamide, and prednisone (schedule B_1), eight patients responded (all with visceral disease) with a median regression duration of 25 weeks (range, 19-45+ weeks). Seven of the eight responders have been in remission status from 19 to 45+ weeks. One of three patients responded to schedule B_1 which was used as secondary treatment.

Six of 12 patients responded to the combination of 5-fluorouracil, cyclophosphamide, prednisone, and calusterone (schedule B_2) used as initial therapy; four of these patients had visceral disease. The median length of regression was 35 weeks (range, 22-45+ weeks) and all patients are still in remission. Two of five patients responded to schedule B_2 given as secondary treatment.

TOXIC EFFECTS

Toxicity data are presented in tables 3 and 4. All toxic effects were mild for schedules B_1 and B_2. Moderate to severe neurotoxicity after the first or second treatment using schedule A caused us to decrease the dose of vincristine from 1.4 mg/m^2 (2.0 mg maximum) on Days 1 and 5 to 1.0 mg/m^2 (1.5 mg maximum) in the last ten patients treated. At the 1.4-mg/m^2 dose level, severe myalgias, anorexia, and muscle weakness were common. At the 1-mg/m^2 level, neurotoxicity was acceptable. Hematologic toxic reactions were seen in 22 of 23 patients receiving schedule A with a median wbc nadir of 2400 cells/mm^3 (range, 900-3700 cells/mm^3) on Day 14. The median time to recovery from the nadir was 5 days. Thrombocytopenia was seen in seven of 23 patients with a median platelet nadir of 70,000 platelets/mm^3 (range, 16,000-83,000 platelets/mm^3) on Day 13. Recovery occurred within 7 days. Dosage reductions of all three drugs occurred commonly. Five of 23 patients had their doses of methotrexate and adriamycin reduced by an average of 25%. Eleven of 23 patients required reduction in the vincristine dose (an average of 50% at the 1.4-mg/m^2 level and 33% at the 1.0-mg/m^2 level). No cardiac toxicity was seen. Other toxic effects (table 4) were as expected and no more than moderate in severity.

Eight of 26 patients receiving schedules B_1 and B_2 had no hematologic toxic reactions while seven of 26 patients had wbc nadirs under 2000 cells/mm^3. On both schedules, the wbc nadir occurred on Day 17 and recovered within 7 days. The median nadir for patients with wbc nadirs < 4100 cells/mm^3 was 2400 cells/mm^3 for schedule B_1 (range, 1100-3300 cells/mm^3) and 1900 cells/mm^3 for schedule B_2 (range, 700-3200 cells/mm^3). Thrombocytopenia occurred in only one patient for each schedule (92,000 and 72,000 platelets/mm^3 respectively). Virilization, usually mild, occurred in five of 12 patients receiving calusterone. Other toxic effects (table 4) were as expected and no more than moderate in severity.

DISCUSSION

This study indicates a 48% response rate (11 of 23 patients) for the combination of vincristine, methotrexate, and adriamycin in previously untreated patients with disseminated breast cancer. This response is no better than the response rates of 50% (ten of 20 patients) for adriamycin used alone (1) and 39% (nine of 23 patients) for the combination of adriamycin and methotrexate (6). None of these adriamycin programs are superior to our "control" treatment using 5-fluorouracil, cyclophosphamide, and prednisone (with or without calusterone) which yielded a 54% (14 of 26 patients) response rate.

TABLE 3.—Hematologic toxicity*

Treatment schedule†	Wbc nadir (cells/mm³)					Platelet nadir (platelets/mm³)		
	>4100	3000-4100	2000-3000	1000-2000	<1000	>130,000	75,000-130,000	<75,000
A	1/23	3/23	11/23	7/23	1/23	17/23	2/23	4/23
B_1	3/14	2/14	5/14	4/14	—	13/14	1/14	—
B_2	6/12	2/12	1/12	1/12	2/12	11/12	—	1/12

*No. of patients with hematologic toxic reaction/No. of patients treated.
†See table 1 for explanation of drug schedules.

TABLE 4.—Toxic effects other than hematologic*

Toxic effect	Schedule†		
	A	B_1	B_2
Nausea	21/23	9/14	9/12
Vomiting	13/23	5/14	1/12
Diarrhea	3/23	1/14	1/12
Stomatitis	7/23	0/14	1/12
Alopecia	23/23	9/14	11/12
Neurologic	23/23	0/14	0/12
Cardiac	0/23	0/14	0/12
Virilization	0/23	0/14	5/12

*No. of patients with toxic reaction/No. of patients treated.
†See table 1 for explanation of drug schedules.

The numbers are too few to calculate meaningful data concerning response duration but it is our feeling that the median duration of response for the adriamycin combination may be longer than for adriamycin alone, possibly because only nine courses of adriamycin alone could be given at the 60-mg/m² level (9×60 mg/m² = 540 mg/m²) versus 13 courses of the combination with adriamycin at 40 mg/m² (13×40 mg/m² = 520 mg/m²).

At present, numbers are again too few to comment upon the value of adding calusterone, a male hormone, to the "control" treatment except to say that no startling differences were seen.

Again, as with adriamycin used alone (1) or combined with methotrexate (6), response to adriamycin in combination with vincristine and methotrexate was extremely poor for patients failing either of the 5-fluorouracil plus cyclophosphamide plus prednisone combination programs.

Overall toxicity for the adriamycin, vincristine, and methotrexate combination was more severe than for either of the other two schedules reported here. The combination with adriamycin produced more consistent hematologic toxicity and much more anorexia, myalgias, and neurologic impairment than we have seen in other programs using the same doses of vincristine initially employed in this study.

Other drug combinations with adriamycin are being studied. One area where adriamycin combinations may be used in the future is in the treatment programs using alternating treatment schedules (eg, alternating adriamycin plus drug X with the combination 5-fluorouracil, cyclophosphamide, and prednisone). At a minimum, effective adriamycin combinations should allow for longer treatment durations than for adriamycin used alone due to the reduction in doses of adriamycin required per course when used in a combination regimen.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8; ADR
Calusterone: NSC-88536; CAS reg. No. 17021-26-0; testosterone, 7β, 17-dimethyl-; CAL
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; CTX
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methy]methylamino]benzoyl]-; MTX
Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,-11,20-trione, 17,21-dihydroxy-; PRED
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); VCR

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European Studies With Adriamycin (NSC-123127) in Lung Cancer[1,2]

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SUMMARY

European clinical trials in lung cancer involving adriamycin used alone or in combination with other agents are briefly reviewed. Adriamycin has clearly shown a definite efficacy against bronchogenic carcinoma apparently without cell-type specificity, yet the survival is not markedly increased. An intermittent high-dose schedule is well tolerated; however, this schedule does not seem to reduce the dose-related cardiac toxic effects. No conclusive data are available with regard to the value of adriamycin in combination with other chemotherapeutic agents and with other treatment modalities.

[Cancer Chemother Rep Part 3, vol 6:343-347, 1975]

Bronchogenic carcinoma remains the most lethal type of cancer and its incidence is still increasing. Chemotherapeutic agents used alone or in combination appear only marginally effective; in fact, within the last decade, the survival rate of patients with bronchogenic carcinoma has not improved substantially. Therefore, focus on the development of new drugs must be stressed.

Since the first clinical studies in Italy (1), adriamycin has been widely investigated (2) and a definite antitumor activity has been demonstrated against a wide range of tumor types, including solid tumors as well as hematologic malignancies.

In this paper, an attempt is made to review the European experience with adriamycin in lung cancer, using this promising drug either as a single agent or as an agent integrated into combined regimens.

SINGLE-AGENT STUDIES

Table 1 summarizes the essential results obtained in phase I and II trials carried out in Europe. In the series initially reported by Bonadonna et al (3), the doses used were 0.4 mg/kg in pretreated patients and 0.6 mg/kg in all other patients. In most cases, the drug was given daily for 3 consecutive days and this course was repeated after a 1-week rest period. In a subsequent study undertaken in cooperation with the Southwest Oncology Group (4), the drug was administered at a dose of 25 mg/m^2 for 3 consecutive days and the courses were repeated every 3 weeks. The overall response rate in a total of 37 patients was 9% (5).

In the first study reported by Praga (6), adriamycin was given in a dosage schedule similar to that used in the initial series of Bonadonna, but it began with a loading-dose schedule and continued with an alternate-day schedule. In a second study, adriamycin was administered at a dose of 1 mg/kg/day for 2 consecutive days, followed after a 21-day interval by courses of 0.5 mg/kg/day for 2 consecutive days repeated every 3 weeks. Of a total of 58 patients, 7% are reported to have achieved an objective response.[6]

In another study conducted by Kenis et al (7) adriamycin was given at a dose of 75 mg/m^2 every 3 weeks. Of 50 patients completing at least two courses, 16 had a partial response for a response rate of 32%.

Despite the relatively high response rate observed in the latter trial, it was suggested that no survival increase was achieved. Among the evaluable patients, the median survival time from the beginning of chemotherapy was 200 days for the responders and 162 days for the nonresponders (table 2). The number of responses was greater and the median survival was longer among the patients who had had no prior therapy. The survival time was the same for the responders and the nonresponders among the patients with prior therapy. This supports the view that responders are usually found among good-risk patients. It is also worth emphasizing that more than half of the patients were not treated prior to entering the trial. This might account at least partially for the relatively good results noted in this series.

There is no obvious difference in the frequency of responses between the different histologic classes

[1]Supported by grant 20187 from the Fonds de la Recherche Scientifique Médicale, Belgium.

[2]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

[3]Service de Médecine et d'Investigation Clinique, Département de Chimiothérapie, Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, Belgium.

[4]We wish to express our gratitude to Drs. C. Praga and P. Alberto for providing recent results regarding their clinical trials and to Dr. O. Karim from Farmitalia Benelux for his assistance in the preparation of this manuscript.

[5]*Reprint requests to:* Dr. M. Rozencweig, Service de Médecine et d'Investigation Clinique, Département de Chimiotherapie, Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, Brussels, Belgium.

[6]Praga C. Personal communication, Dec 1974.

(table 3). In the combined group of patients reported by Bonadonna et al (5) and by Kenis et al (7), 11 remissions were observed among 39 patients with epidermoid carcinoma and five remissions among 19 patients with small cell carcinoma.

Based on pharmacologic investigations (8,9) and the clinical observations of fewer toxic effects, especially regarding stomatitis, an intermittent single high-dose schedule is now widely used without apparent loss of therapeutic effectiveness (table 4). With a dose schedule of 75 mg/m^2 every 3 weeks, alopecia is seen in virtually all patients. Gastrointestinal toxic effects evidenced by nausea, occasional vomiting, and diarrhea may occur but rarely limit the drug's clinical use. Hematologic toxic effects are usually not severe and are almost always reversible within 3 weeks. In the series reported by Kenis et al (7), severe leukopenia with less than 1000 cells/mm^3 occurred in four of 30 evaluable patients and platelets fell below 30,000 platelets/mm^3 in three of 29. There were no cumulative hematologic toxic effects. Finally, cardiac toxicity is essentially dose dependent and at present it remains difficult to assess accurately whether this side effect is also schedule dependent.

TABLE 1.—Adriamycin in lung cancer: European phase I-II studies

Investigator (ref No.)	Dose schedule	No. of patients with >50% response/ total No. of evaluable patients
Bonadonna (5)	0.4 mg/kg/day × 3, repeated after 1 wk	0/10
	0.6 mg/kg/day × 3, repeated after 1 wk	2/9
	25 mg/m^2/day × 3, q3wks	2/18
		4/37
Praga (see footnote 6)	0.5 mg/kg/day × 3, repeated after 1 wk	3/41
	1 mg/kg/day × 2 + 0.5 mg/kg/day × 2, q3wks	1/17
		4/58
Kenis (7)	75 mg/m^2, q3wks	16/50

TABLE 2.—Median survival in patients with lung cancer*†

	Median survival (days) in patients with—‡		
	No prior therapy	Prior therapy	Total
Responders	220 (11)	112 (8)	200 (19)
Nonresponders	190 (12)	112 (19)	162 (31)
Totals	210 (23)	112 (27)	169 (50)

*Nonevaluable cases have been excluded.
†Originally published in *Eur J Cancer* (8:487, 1972).
‡Nos. in parentheses = No. of patients.

TABLE 3.—Adriamycin in the treatment of lung cancer: response by cell type

Cell type	No. of responses/ total No. of patients treated		
	Kenis (ref 7)	Bonadonna (ref 5)	Total
Epidermoid carcinoma	11/30	0/9	11/39
Small cell carcinoma	3/14	2/5	5/19
Anaplastic large cell carcinoma	2/2	2/11	4/13
Glandular carcinoma	0/2	0/6	0/8
Unclassified carcinoma	0/2	0/6	0/8
Totals	16/50	4/37	20/87

COMBINATION CHEMOTHERAPY STUDIES

Combination chemotherapy with adriamycin and methotrexate in lung cancer has been studied recently by Praga[7] (table 5). In a first phase I trial, adriamycin (40 mg/day) given intravenously (iv) for 2 consecutive days was followed by methotrexate (10 mg/day) given intramuscularly from the third to the seventh day. The courses were repeated every 3 weeks. In a second phase I trial, adriamycin (1 mg/kg/day) and methotrexate (50 mg/day) were given iv on Day 1 every 3 weeks. In both trials, prednisone might be given at a maximum dose of 20 mg/day. Poor response rates were observed in both trials. However, although gastrointestinal toxic effects were severe and frequent when these drugs were administered successively, a simultaneous combination given intermittently was seemingly well tolerated, encouraging the prospect of higher doses in further studies.

The Swiss Group for Clinical Cancer Research has recently completed a randomized trial in bronchogenic carcinoma with two combination regimens.[8] In the study group, the patients were treated with sequential combination chemotherapy according to Schabel's hypothesis (10). They received three courses starting with cell-cycle-nonspecific agents, ie, CCNU (70 mg/m^2), adriamycin (30 mg/m^2), cyclophosphamide (70 mg/m^2/day), and procarbazine (70 mg/m^2/day), followed by cell-cycle-specific agents, ie, vincristine (1.2 mg/m^2/wk), methotrexate (25 mg/m^2/wk), and hydroxyurea (1 g/m^2 × 3/wk). In the control group, the patients were treated with a simultaneous combination of methotrexate (25 mg/m^2/wk), vincristine (1.2 mg/m^2/wk), cyclophosphamide (70 mg/m^2/day), and procarbazine (70 mg/m^2/day). After 24 weeks, the responding patients and the patients in the no-change category were randomized in two groups for maintenance therapy (fig 1).

There were 104 evaluable patients after the first 8 weeks of the induction phase of the treatment (table 6). Objective response rates in the study group and in the control group were 39% and 44% respectively. A significant difference in responsiveness between small cell and epidermoid carcinoma may be noted in both treatment groups, especially in the study group where the response rate in small cell carcinoma was 81%. No impressive difference in the actuarial survival curves was obtained between the treatment groups. However, in the study group, two patients with small cell carcinomas are still living, 100 weeks from the onset of their sequential treatment (fig 2).

[7] See footnote 6.
[8] Alberto P. Personal communication, Nov 1974.

TABLE 4.—Dose schedule-related stomatitis occurring after adriamycin therapy

Dose schedule	Investigator (ref No.)	No. of patients with stomatitis/No. treated
0.4-0.6 mg/kg/day × 3, repeated after 1 wk	Praga (6)	19/41
	Bonadonna (3)	11/14
		30/55 (54%)
75 mg/m^2/day, q3wks	Kenis (7)	2/50 (4%)

TABLE 5.—Combination chemotherapy with adriamycin and methotrexate

Drugs	Dose schedule	Total No. of patients	Response rate (%)	Gastrointestinal toxic effects (%)		
				Stomatitis	Diarrhea	Other
Adriamycin + methotrexate	40 mg/day iv, Days 1 and 2 10 mg/day im, Days 3-7	21	5	50	27	27
Adriamycin + methotrexate	1 mg/kg/day iv, Day 1 50 mg/day iv, Day 1	19	5	16	11	32

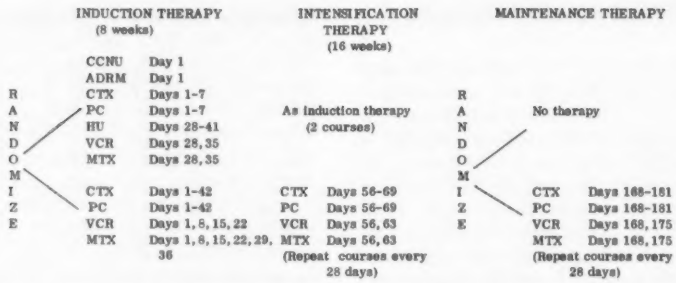

FIGURE 1.—Sequential treatment with cell-cycle-nonspecific agents and cell-cycle-specific agents compared with simultaneous combination chemotherapy in bronchogenic carcinoma. Drugs used were: CCNU = 70 mg/m², adriamycin (ADRM)= 30 mg/m², cyclophosphamide (CTX) =70 mg/m²/day, procarbazine (PC) =70 mg/m²/day, hydroxyurea (HU) = 1 g/m² × 3/wk, vincristine (VCR) = 1.2 mg/m²/wk, and methotrexate (MTX) =25 mg/m²/wk. (Data of the Swiss Group for Clinical Cancer Research.)

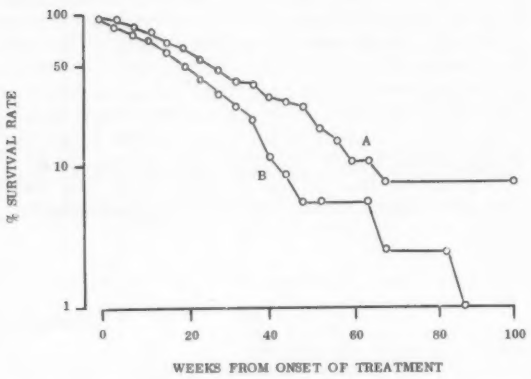

FIGURE 2.—Actuarial survival curves for patients with bronchogenic carcinoma treated with sequential combination chemotherapy (A) or simultaneous combination chemotherapy (B). (Data of the Swiss Group for Clinical Cancer Research.)

The EORTC Cooperative Group for Clinical Trials in Lung Cancer is presently studying the combination of adriamycin (45 mg/m²) and methotrexate (40 mg/m² iv) given simultaneously every 3 weeks in patients with small cell carcinoma who are in relapse after radiation therapy and combination chemotherapy with other drugs (fig 3). This trial is still in progress and it is too early to evaluate the preliminary results.

The Swiss Group for Clinical Cancer Research and the EORTC Cooperative Group for Clinical Trials in Lung Cancer will be working together in future studies in bronchogenic carcinoma. In both cooperative groups, randomized clinical trials will be carried out independently but with a common treatment group. This procedure will ensure more meaningful analysis of results achieved by different cooperative groups. Moreover, a comparison will become theoretically possible between all treatment groups of both cooperative studies which will result in an appreciable time gain.

TABLE 6.—CCNS-CCS* sequence vs simultaneous combination chemotherapy†

Treatment	Disease	Total No. of patients	No. of evaluable patients	No. of responders (%)
CCNS-CCS sequence	Small cell carcinoma	27	26	21 (81)
	Large cell carcinoma	10	7	0
	Epidermoid carcinoma	20	19	2 (11)
	Adenocarcinoma	10	7	0
		67	59	23 (39)
Simultaneous (4 drugs)	Small cell carcinoma	25	22	15 (68)
	Large cell carcinoma	6	4	0
	Epidermoid carcinoma	18	16	4 (25)
	Adenocarcinoma	6	3	1
		55	45	20 (44)

*CCNS = cell-cycle-nonspecific agents; CCS = cell-cycle-specific agents.
†Data of the Swiss Group for Clinical Cancer Research.

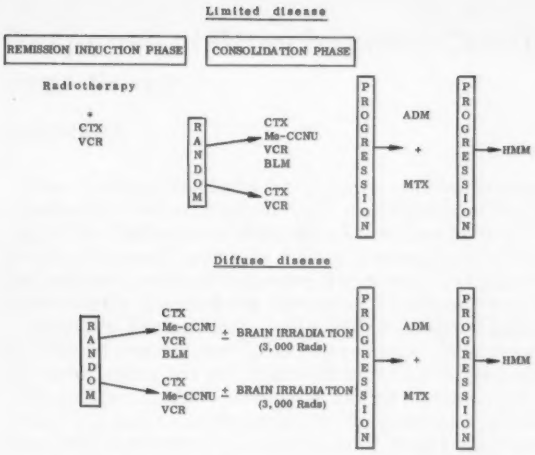

FIGURE 3.—Outline of study for untreated small cell bronchogenic carcinoma. Drugs used are: cyclophosphamide (CTX), vincristine (VCR), methyl-CCNU (Me-CCNU), bleomycin (BLM), adriamycin (ADM), methotrexate (MTX), and hexamethylmelamine (HMM). (Data of the EORTC Cooperative Group for Clinical Trials in Lung Cancer.)

CONCLUSION

Adriamycin administered alone is obviously of clinical value in advanced bronchogenic carcinoma at least as far as direct antitumor effect is concerned. It remains to be proven that adriamycin can increase survival rates. No firm conclusion can be drawn concerning a possible cell-type specificity. However, the EORTC data suggest that it might be more active in epidermoid carcinoma. An intermittent high-dose schedule is strongly advocated, especially with respect to the low incidence of stomatitis, without any apparent reduction in the response rate.

Much more data are needed before any evaluation of the drug in combinations with other chemotherapeutic agents or with other treatment modalities can be made. Combination studies including adriamycin must be actively pursued in an attempt to delay the dose-related cardiac toxicity and to prolong the usual short-lasting response induced by this promising drug.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
Bleomycin: NSC-125066
CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Hexamethylmelamine: NSC-13875; CAS reg. No. 645-05-6
Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-
Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,-11,20-trione, 17,21-dihydroxy-
Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl- α-(2-methylhydrazino)-, monohydrochloride
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Response of Bronchogenic Carcinoma to Adriamycin (NSC-123127)[1]

Oleg S. Selawry[2,3,4]

SUMMARY

Four hundred and forty-five patients with microscopically proven bronchogenic carcinoma received adriamycin in single doses of 60-75 mg/m² or in doses of 15-35 mg/m² on 3 subsequent days. Repeat courses up to a total dose of 450-500 mg/m² were given at 3-4-week intervals. Tumor shrinkage of 50% or more occurred in 18% of 377 patients with evaluable disease. The highest response rates, 32% and 37%, occurred where single spaced doses were used. Previously untreated patients were the best responders. Performance status had no apparent influence on response. Small-cell carcinoma was the most sensitive cell type with a response rate of 28% for 64 patients including five patients with complete tumor regression. Patients with large-cell carcinoma, adenocarcinoma, and epidermoid carcinoma had response rates of 16%, 15%, and 14%, respectively. Responders lived from two to four times longer than nonresponders. It is concluded that adriamycin is useful for palliation of patients with disseminated lung cancer.

[Cancer Chemother Rep Part 3, vol 6:349-351, 1975]

During the short time since it has become available, adriamycin has assumed a prominent place in the treatment of lung cancer. With a combined total of 377 evaluable patients (among a total of 445 patients treated), it has become the fifth most commonly used single drug after such well established agents as cyclophosphamide, mechlorethamine, hexamethylmelamine, and methotrexate (table 1).[5] To what extent is this interest a spillover from the impressive responses of other tumors and what is the place of adriamycin as a single agent for palliative treatment of bronchogenic carcinoma?

The overall objective response rate of 10% for adriamycin falls within the range of responses to other single agents (1). This estimate is based on tumor regressions of 50% or more, using the product of the longest times the widest perpendicular diameters in the absence of progression or occurrence of new lesions elsewhere (2).

The overall response rate of 18% includes individual response rates between 8% and 37% in reported studies of more than 25 patients (3-8).[6] The smaller studies amplify this difference to 0-50% (as shown in table 1) probably by sampling error (9-11). There is reasonable evidence that the difference in response rate was due to three major factors: patient selection, drug dose, and cell type of bronchogenic carcinoma.

TABLE 1.—Objective response of bronchogenic carcinoma to the 5 most commonly used single agents

Drug	No. of evaluable patients	Response* (%)/ range in individual studies
Cyclophosphamide	1513	20/0-63
Mechlorethamine	1442	31/0-68
Hexamethylmelamine	485	18/10-30
Methotrexate	416	22/3-43
Adriamycin	377	18/0-50

*Tumor shrinkage of 50% or more.

PATIENT SELECTION

The study with the *lowest* response rate (8% [8]) included 168 patients. Of these patients, 75% have had prior chemotherapy and 66% have had prior radiotherapy; 63% were bedridden. This implies an adverse effect from prior myelosuppressive treatment and/or from poor performance. Indeed, three out of four studies show substantially higher response rates in patients who have had no prior

[1]Adriamycin: CAS reg. No. 23214-92-8.

[2]Division of Pulmonary Oncology, Department of Oncology, University of Miami School of Medicine, Fla.

[3]Part of this work was done during the author's tenure with the National Cancer Institute-Veterans' Administration (NCI-VA) Medical Oncology Branch, VA Hospital, Washington, DC.

[4]*Reprint requests to:* Oleg S. Selawry, MD, Division of Pulmonary Oncology, Department of Oncology, University of Miami School of Medicine, 1400 NW 10th Ave, Miami, Fla 33152.

[5]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard.

Hexamethylmelamine: NSC-13875; CAS reg. No. 645-05-6.

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

[6]Benjamin RS, and Ojudinrin O. Baltimore Cancer Research Center, DCT, NCI, Md, and Department of Oncology, University of Southern California, Los Angeles. Personal communications.

TABLE 2.—Prior treatment and objective response*

Patients responding (%) with—		
Prior treatment	No prior treatment	Ref No.
22	67	Footnote No. 6
31	28	6
30	48	7
6	12	8

*Including chemotherapy and radiotherapy, excluding surgery.

TABLE 3.—Performance and objective response†

Performance status*	Response (%)	No. of patients treated
Good	29	17
Medium	33	18
Poor	43	16

*Karnofsky scale: good = 70-100; medium = 50-60; poor = <50.
†See footnote No. 6.

treatment compared to their treated counterparts (table 2). It is surprising, however, that performance status has no apparent bearing on objective response (table 3) in the one study where data are accordingly tabulated.[6] Larger numbers of patients would be desirable before more definite conclusions could be reached. Therefore, untreated patients, or those with only short courses of prior myelosuppressive therapy, have a better chance to respond to adriamycin than patients with compromised organ functions.

DRUG DOSE

Adriamycin was usually given in 3-day courses or in single doses every 3-4 weeks. A total dose of approximately 500 mg/m^2 body surface area was not exceeded because of the well-known risk of myocardial toxicity at higher cumulative doses and because of the uncertainty of whether prior radiotherapy to the mediastinum (including the heart) might increase the risk of cardiotoxicity. The dose for the 3-day treatment course varied between 45 and 105 mg/m^2. Single spaced doses ranged from 60 to 75 mg/m^2 given intravenously (iv).

The two studies with single spaced doses of 60 and 75 mg/m^2, respectively, showed the highest response rates followed by the seven studies with 3-day treatment courses (table 4). In the absence of prospective controlled clinical trials, it appears that single spaced doses of adriamycin are twice as effective as equivalent subdivided doses and are at least as effective as 3-day courses using higher equivalent doses. Thus, a more therapeutic effect might be gained at lower cumulative doses with the possibility of a lower risk of cardiotoxicity. It is suggested, therefore, that starting doses of 75 mg/m^2 be considered for patients with uncompromised bone marrow function and doses of 60 mg/m^2 be considered for patients with prior myelosuppression from drugs or radiotherapy. Repeat doses are given as toxicity clears, at approximately 3-week intervals, and are increased or decreased depending on individual tolerance.

CELL TYPE

Responses were reported by cell type for all patients except 28 (table 5). Small-cell carcinoma (in-

TABLE 4.—Dose, schedule, and response

Dose (mg/m^2 q3wks)		Response (%)	Ref No.
Per course	Per injection		
60	60	37	Footnote No. 6
75	75	32	7
60-90	20-30	30*	9,11
90-105	30-35	29	6
60-75	20-25	15	5
66-75	22-25	14	3,4
60	20	8	8
45	15	0	3,4

*Two small studies of 4 and 6 patients, respectively.

TABLE 5.—Response to adriamycin by cell type

Cell type	Total No. of patients—		Response (%)
	Evaluable	Responding	
Small-cell	64	18 (5)*	28
Large-cell	64	10	16
Adenocarcinoma	99	15	15
Epidermoid	125	18 (1)*	14
Unclassified	28	2	7

*Nos. in parentheses = complete regressions.

TABLE 6.—Survival and response

Type of response	Median survival time (mos) in study described in ref No.—			
	*	6	7	8
None	2	3	5.4	2.5
Static disease	5	10.5	—	—
Objective	8	8.5	6.7	6

*See footnote No. 6.

cluding the oat-cell variety) was the most responsive tumor, including complete regressions in five of 18 responders, followed by large-cell carcinoma, adenocarcinoma, and epidermoid carcinoma (one complete regression).

Thus, adriamycin ranks seventh among 17 drugs which have been tested against small-cell carcinoma, sixth in adenocarcinoma and large-cell carcinoma, and tenth in epidermoid carcinoma (12).

ONSET AND DURATION OF RESPONSE

Objective tumor regression was usually noted within 3-6 weeks (8). Later responses were uncommon. The median duration of response varied between 2 months for the 13 responders receiving lower doses and extensive prior treatment (8) and 5 and 7 months for the ten and nine responders, respectively, among patients with single spaced doses (7)[7] and less extensive prior treatment.

Responders lived for a median of 6-8.5 months (table 6) or two to four times longer than nonresponders (with the exception of one study where the differences were unimpressive [7]).

CONCLUSION

Adriamycin is a valuable addition to the armamentarium of the oncologist for temporary palliation of metastatic bronchogenic carcinoma. Single spaced doses of 60-75 mg/m^2 given at 3-week intervals might be more effective than equivalent doses given on 3 consecutive days. Best responses are noted in patients with small-cell carcinoma. Adriamycin might best be used in combination chemotherapy because (*a*) other single agents are more active for each respective cell type; (*b*) the anthracycline is apparently less effective when given after prior myelosuppressive therapy; and (*c*) drug combinations including adriamycin showed more than an additive effect for treatment of small-cell carcinoma (12).

[7] See footnote 6.

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Polychemotherapy With Adriamycin (NSC-123127) in Bronchogenic Carcinoma[1]

Oleg S. Selawry[2,3,4]

SUMMARY

Combination chemotherapy including adriamycin shows promise for the palliative treatment of small-cell carcinoma, with tumor shrinkage of 50% or better in 65 (77%) of 84 patients included in four studies. Response rates for large-cell carcinoma (61% of 23 patients, including 22% complete regressions) and for epidermoid carcinoma (42% of 50 patients in the best study) are of potential interest pending further evaluation. No response was reported for 17 patients with adenocarcinoma.

[Cancer Chemother Rep Part 3, vol 6:353-359, 1975]

Adriamycin shows definite activity in the four major types of lung cancer (1) as measured by an objective tumor regression of 50% or better (2) and by the prolonged survival of patients with objective tumor regression when compared to nonresponders (1). The drug appears to be most effective in patients without prior treatment (1), yet for each cell type there are other drugs with apparently greater efficacy (tables 1-4). Hence, this anthracycline might best be used in combination with other drugs. Lack of cross-resistance with most other agents of similar or higher efficacy in bronchogenic carcinoma (for supporting data *see* other articles in this issue) makes combination chemotherapy particularly attractive. Moreover, polychemotherapy results in lower cumulative doses of adriamycin and hence decreases the risk of life-threatening cardiotoxicity. It is therefore not surprising to find 25 recorded studies[5] where adriamycin has been combined with other drugs for the treatment of cancer. Data on nine of these studies have been published (3-10)[6] (mostly as abstracts) and these reports form the substance of this review.

CONTROLLED CLINICAL TRIALS

The first of the three controlled clinical trials listed in table 5 shows a statistically significant superiority for a three-drug combination including adriamycin, NSC-45388, and cyclophosphamide over cyclophosphamide used alone in small-cell carcinoma (3) based on a preliminary evaluation of 50% of the patients entered into the study. The response rate of 77% for this drug combination is better than the expected response rates for each of the component agents alone. The response rate of 17% for cyclophosphamide alone is lower than expected (table 3). These results should be of clinical importance if confirmed by the final evaluation.

The second controlled clinical trial (table 5) shows that the three-drug combination including adriamycin, mechlorethamine, and methotrexate is equal in effectiveness to the same three drugs followed by a mutually non-cross-resistant combination of 5-fluorouracil, hydroxyurea (both cell cycle phase-sensitive drugs), and procarbazine (4). The overall response rate of 23% in each of the study arms is unimpressive. The three-drug combination includes two patients with complete tumor regression. The median duration of response for the three responders in each of the study arms favors the three-drug combination (5+ months) over the six-drug combination (2.5+ months).

Use of the third controlled clinical trial (5) was also motivated by cell kinetic considerations. The experimental arm includes a combination of four cell cycle phase-nonsensitive drugs: adriamycin, CCNU, cyclophosphamide, and procarbazine. Four weeks later, when the tumor is expected to grow again, the patients receive three cell cycle phase-sensitive agents (hydroxyurea, methotrexate, and vincristine) as outlined in table 5 (study 3A). The control arm represents a "standard" of proven value to the investigators of this study and includes cyclophosphamide, methotrexate, procarbazine, and vincris-

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[1]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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[5]Registered at the International Association for Study of Lung Cancer by L. Broder and O. Selawry, in close cooperation with S.K. Carter, Deputy Director, Division of Cancer Treatment, NCI, Bethesda, Md.

[6]Einhorn LH, Farber MO, Fee WH, et al. Bleomycin, adriamycin, Cytoxan, and Oncovin combination chemotherapy in oat cell lung cancer. Presented at a meeting of the American College of Physicians, Apr 1975.

TABLE 1.—Objective response of adenocarcinoma to adriamycin as compared with other agents

Rank	Drug	No. of patients treated	Response rate (%)	Ref No.
1	Methotrexate	25	32	11
2	Mechlorethamine	86	28	11
3	Mitomycin C	11	27	11
4	Cyclophosphamide	50	21	11,13
5	Procarbazine	16	19	11
6	**Adriamycin**	**99**	**15**	**1,11**
7	Hexamethylmelamine	39	15	11
8	Bleomycin	30	13	11
9	CCNU	55	13	11
10	Methyl-CCNU	83	10*	11,14
11	Dibromodulcitol	12	8	11
12	5-Fluorouracil	19	5	11,13
13	Busulfan	28	4	11
14	BCNU	22	0	11
15	Carbazilquinone	11	0	11

*Including complete regression among 6 patients with bronchiolar carcinoma.

TABLE 2.—Objective response of large-cell carcinoma to adriamycin as compared with other agents

Rank	Drug	No. of patients treated	Response rate (%)	Ref No.
1	Procarbazine	17	35	11
2	Mechlorethamine	148	26	11
3	Methyl-CCNU	43	21	11,14
4	Hexamethylmelamine	39	18	11
5	CCNU	41	17	11
6	**Adriamycin**	**64**	**16**	**1,11**
7	Cyclophosphamide	37	13	11,13
8	Methotrexate	104	12	11
9	BCNU	22	9	11
10	6-Mercaptopurine	29	3	11
11	Colchicine	12	0	11
12	5-Fluorouracil	11	0	11,13

TABLE 3.—Objective response of small-cell carcinoma to adriamycin as compared with other agents

Rank	Drug	No. of patients treated	Response rate (%)	Ref No.
1	1-Methyl-1-nitrosourea	26*	65	11
2	Methotrexate	79	39	11
3	Mechlorethamine	80	39	11
4	Vincristine	24	33	11,12
5	Cyclophosphamide	300	31	11,13
6	Hexamethylmelamine	71	31	11
7	**Adriamycin**	**64**	**28**	**1,11**
8	Procarbazine	44*	25	11
9	BCNU	19	21	11
10	Dibromodulcitol	15*	20	11
11	NSC-141540	10	20	11
12	CCNU	31	19	11
13	Busulfan	17	18	11
14	Dehydroemetine	19	11	11
15	Methyl-CCNU	39	10	11,14
16	Mitomycin C	11	9	11
17	Bleomycin	27	0	11

*Includes large-cell anaplastic carcinoma.

TABLE 4.—Objective response of epidermoid carcinoma to adriamycin as compared with other agents

Rank	Drug	No. of patients treated	Response rate (%)	Ref No.
1	Methotrexate	140	25	11
2	Dibromodulcitol	39	23	11
3	Dehydroemetine	9	"22"	11
4	Mechlorethamine	183	21	11
5	Cyclophosphamide	242	20	11,13
6	CCNU	58	17	11
7	Vinblastine	20	16	11
8	5-Fluorouracil	20	15	11,13
9	1-Methyl-1-nitrosourea	14	15	11
10	Adriamycin	125	14	1,11
11	Bleomycin	265	13	11
12	Methyl-CCNU	112	13	11,14
13	Procarbazine	55	13	11
14	Hexamethylmelamine	68	12	11
15	BCNU	34	6	11
16	6-Mercaptopurine	16	0	11
17	Mitomycin C	22	0	11

tine (table 5, 3B). There is evidence for superiority of the experimental arm over the "standard" arm for a subgroup of patients, based on superior survival which reaches statistical significance within 270 days after the start of treatment. This subgroup might well include small-cell carcinoma rather than other cell types, or "responders" as compared to "nonresponders," because of the impressively lower response rates for adenocarcinoma and epidermoid carcinoma in the experimental arm (3A). This becomes more apparent upon inclusion of historical controls (6) into the data for the "standard" arm (3B).

UNCONTROLLED TRIALS

The data on the six uncontrolled clinical trials are summarized in table 6 in sequence of response rates of the most sensitive tumor type. The results tend to confirm some promise for small-cell carcinoma and possibly for epidermoid carcinoma. The list is led (7) by the predecessor study to the first randomized study listed in table 5 (3), using the same three drugs for initiation of treatment followed by two cell cycle phase-sensitive drugs (hydroxyurea and vincristine) on Days 22-24 of each treatment cycle. Tumor regression in 12 of 13 patients with large- and small-cell carcinoma (breakdown not specified) looks more impressive than in the controlled study of table 5, because of the inclusion of at least four patients with large-cell carcinoma and because five of the 12 responders experienced complete tumor regressions. Similarly promising results were obtained with a combination of adriamycin, cyclophosphamide, bleomycin, and vincristine.[7]

The only study with possible promise for epidermoid carcinoma (8) includes the combination of bleomycin, adriamycin, CCNU, vincristine, and mechlorethamine (BACON). The combinations with apparent promise for small- and large-cell anaplastic carcinoma (6,7) show no advantage for epidermoid carcinoma. None of the four patients with adenocarcinoma responded (7,9). Evaluation of response to a combination of adriamycin with cyclophosphamide, 5-fluorouracil, and methotrexate (10) must await larger patient numbers. The combination of adriamycin with bleomycin (10) shows no apparent advantage over adriamycin alone in a pilot study.

[7] See footnote 6.

DISCUSSION OF RESULTS

The therapeutic effect of polychemotherapy with adriamycin can be gauged by the efficacy of the respective treatment regimens when compared to monochemotherapy using the best component drugs and to other drug combinations.

Efficacy will be arbitrarily defined as objective tumor regression (2) because of the generally good correlation between objective regression and survival (2,11). The evidence will be mostly suggestive rather than conclusive because of the limited number of prospective comparative clinical trials, because of the small number of patients in most of the studies, and because of the paucity of quantitative data on important factors of patient selection and response (such as ambulation and symptomatic improvement, treatment-related morbidity, and need for more frequent followup). The same limitation of data prohibits the application of standard pharmacologic principles to the evaluation of polychemotherapy with adriamycin. It is understood that the paucity of data is unavoidable for communications in abstract form.

The most extensive information is available for small-cell carcinoma. Here, adriamycin ranks seventh among single agents with tumor regression in 28% of 64 patients (table 3). This rank is very approximate, because the ranking is based on a review of all available published data (1, 12-14), admitting historical "controls" with their well-recognized variability (2). Nevertheless, there is a statistically

TABLE 5.—Combination chemotherapy with adriamycin—controlled clinical trials

Trial No.	Drugs	Dose (mg/m²)* and schedule	No. of patients— Entered	No. of patients— Evaluated	No. of responses (%)	Cell type	Ref No.
1A	Adriamycin NSC-45388 Cyclophosphamide	50 250 500 } q3wks	26	13	10 (77)*	Small-cell	3
1B	Cyclophosphamide	1100, q3wks	25	12	2(17)*		
2A	Adriamycin Mechlorethamine Methotrexate	40 6 30 } q2wks		4 2 1 2 4 13	0 (0) 1 (50)† 1 (100)† 1(50) 0 (0) 3 (23)	Adenocarcinoma Epidermoid Large-cell Small-cell Unclassified	4
2B	Adriamycin Mechlorethamine Methotrexate 5-Fluorouracil Hydroxyurea Procarbazine	40, Day 1 6, Day 1 30, Day 1 300, orally Days 15,17,19 1500, orally Days 15,17,19 100, orally Days 15-29		2 5 3 3 13	0 (0) 0 (0) 2 (67) 1 (33) 3 (23)	Adenocarcinoma Epidermoid Large-cell Unclassified	
3A	Adriamycin CCNU Cyclophosphamide Procarbazine	30, Days 1,56 70, Days 1,56 70, orally Days 1-7,56-63 70, orally Days 1-7,56-63	10 20 10 27 67	7 19 7 26 59	0 (0) 2 (11) 4 (57) 21 (81) 23 (39)‡*	Adenocarcinoma Epidermoid Large-cell Small-cell	5
	Hydroxyurea Methotrexate Vincristine	1000, orally Days 29,31,33,36,40 25, Days 29, 36 1.2, Days 29,36					
3B	Cyclophosphamide Methotrexate Procarbazine Vincristine	70, orally daily 25, weekly 70, orally daily 1.2, daily	6 18 6 25 55	3 16 4 22 45	1 ("33") 4 (25) 0 (0) 15 (68) 20 (44)§*	Adenocarcinoma Epidermoid Large-cell Small-cell	
3B	Historical controls		8 58 19 59 144	7 46 16 53 122	5 ("71") 13 (28) 8 (50) 33 (62) 59 (48)	Adenocarcinoma Epidermoid Large-cell Small-cell	6
3B	Combined totals		14 76 25 84 199	10 62 20 75 167	6(60) 17 (27) 8 (40) 48 (64) 79 (47)	Adenocarcinoma Epidermoid Large-cell Small-cell	5,6

*Difference statistically significant ($P < 0.05$).
†Complete tumor regression.
‡25% Surviving 280+ days.
§10% Surviving 280+ days.

significant superiority in a prospective clinical trial for at least one three-drug combination (table 1, 1A) over cyclophosphamide, which ranks slightly above adriamycin as a single agent. Three other combinations (table 5 [3A]; table 6 [1 and 2]) have two to three times higher objective response rates than expected for adriamycin alone and for the other single component drugs of each respective combination. This indicates the superiority of adriamycin in combination with certain other drugs over the respective component drugs as single agents.

Only one single agent (1-methyl-1-nitrosourea) might have antitumor activity against small-cell carcinoma similar to that of the drug combinations (table 3). This drug is currently under investigation in combination with cyclophosphamide (16) but not yet with adriamycin.

Comparison of drug combinations with or without adriamycin in small-cell carcinoma shows the four combination regimens with adriamycin in the first four ranks (table 7). The small number of patients in each of these four studies precludes conclusions as to superiority of these drug combinations over the lower ranking treatment programs.

Adriamycin, as a single agent, is much less effective in the other cell types, with objective response rates below 20%. This is exemplified by epidermoid carcinoma, where adriamycin takes tenth rank

TABLE 6.—Uncontrolled studies of adriamycin in combination with other drugs

Drugs	Doses (mg/m²) and schedule	No. of patients: Evaluated	No. of patients: Responding (%)	Cell type	Ref No.
Adriamycin Cyclophosphamide NSC-45388 Hydroxyurea Vincristine	40, monthly 600, monthly 250, monthly 2000, Days 22,24 2, Days 22,24	8 13 2	1 (12) 8 (92) 4 0 (0)	Epidermoid Small-cell Large-cell Adenocarcinoma	7
Adriamycin Bleomycin Cyclophosphamide Vincristine	50, q3wks 15, Day 2, then weekly × 6 750, q3wks 1, Day 2, 6 hrs before bleomycin then weekly × 6	29	22 (76)	Small-cell	8
Adriamycin CCNU Hydroxyurea	20, × 2 days 80 1000, × 7 days orally	9 5 5	1 (11) 3 (60) 3 (60)	Epidermoid Small-cell Large-cell	9
Adriamycin Bleomycin CCNU Mechlorethamine Vincristine	40, q4wks 30, Day 2 as above 65, q8wks 8, q4wks 1, Day 2 as above	50	21 (42)	Epidermoid	9
Adriamycin Cyclophosphamide 5-Fluorouracil Methotrexate	30, q3wks 100, daily orally 100, weekly 10, weekly	4 2 1	1 (25) 0 (0) 0 (0)	Epidermoid Adenocarcinoma Large-cell	10
Adriamycin Bleomycin	20, × 3 days 15-20, × 5 days	15	3 (20)	Not stated	11

TABLE 7.—Ranking of drug combinations for treatment of small-cell carcinoma

Rank	Drugs	Response rate (%)	No. of patients	Randomized?	Ref. No.
1	Adriamycin, cyclophosphamide, NSC-45388, hydroxyurea, vincristine	92	13*	No	7
2	Adriamycin, cyclophosphamide, CCNU, procarbazine, hydroxyurea, vincristine, methotrexate	81	26	Yes	5
3	Adriamycin, cyclophosphamide, NSC-45388	77	13	Yes	3
4	Adriamycin, cyclophosphamide, bleomycin, vincristine	76	29	No	8
5	Cyclophosphamide, methotrexate, vincristine, procarbazine	68 62	22 53	Yes Yes	5 6
6	Cyclophosphamide, CCNU, methotrexate	57	31	Yes	17
7	Cyclophosphamide, CCNU	45	83	Yes	18
8	Cyclophosphamide, methotrexate	31	23	Yes	17

*Includes at least 4 patients with large-cell anaplastic carcinoma.

among 17 drugs studied (table 4). It is surprising, therefore, to find at least one (uncontrolled) study with possible superiority of a drug combination of adriamycin, bleomycin, CCNU, mechlorethamine, and vincristine (table 6 [4]) over single agents.

Combinations including adriamycin rank second and fifth among other drug combinations in epidermoid carcinoma (table 8). Only the two highest ranking combinations in table 8 bear some promise of superiority over single agents. The third-ranking combination has the same activity as the best single drugs. The remaining five drug combinations rank below adriamycin alone and below the best of the two other component agents as single drugs.

Adriamycin ranks as the sixth most effective drug for treatment of adenocarcinoma (table 1) and large-cell carcinoma (table 2). These two cell types were insufficiently exposed to combination chemotherapy with adriamycin; at present, large-cell carcinoma shows better responses to polychemotherapy with adriamycin, including 14 responders (five of them with complete regression) among 23 patients exposed to six different combinations, as compared to no responses among 17 patients with adenocarcinoma exposed to five different drug combinations (tables 5 and 6).

The rationale for using the above drug combinations was based on several widely held concepts, both clinical and experimental. There is a clinical precedent for at least an additive effect of independently active drugs when used for the induction of response in such divergent diseases as acute leukemia in children, the major types of malignant lymphomas, and breast cancer. This concept was applied in all but one of the published studies and in 21 of all 25 current studies for combination chemotherapy in lung cancer. Tables 1-4 supply baselines for possible improvement.

As the number of drugs used simultaneously increases, so too does the number of necessary reductions in individual drug doses. This fact obviates, in part, the possible advantages of combination chemotherapy. This problem is ameliorated by the combination of drugs with divergent toxicity, especially agents with little marrow toxicity such as vincristine and bleomycin. This concept was included in ten of the 25 studies on record. Interestingly, effectiveness of vincristine is only established for small-cell carcinoma and bleomycin has definite activity in less than 20% of patients with all but small-cell carcinoma where it is almost inactive.

Indeed, the inclusion of bleomycin plus vincristine was based on cell kinetic considerations (8)[8] which were the best available at the time of the study design (20), but which might have to be changed in the light of newer findings (21). Other kinetic reasoning went into the sequential use of cell cycle phase-nonspecific agents followed by phase-sensitive drugs at times of expected cell proliferation. Cell kinetic reasoning figured in 12 of the 25 combination studies. Improved study designs might be advanced empirically, Major improvements might have to wait for more extensive cell kinetic data, both clinical and experimental.

Combination of cyclophosphamide (or other alkylating agents) with a nitrosourea was based on data showing more than additive effect in murine tumor systems (22). Moreover, nitrosoureas are convenient for combinations because of their well-known delay of marrow toxicity. This concept was utilized in four studies.

Rationales for other drug combinations have been widely advanced in the literature and elsewhere in this issue. Surprisingly little attention has been given to experimental models on optimal dose ratios for polychemotherapy (23,24). Insufficient clinical data

[8] See footnote 6.

TABLE 8.—Ranking of drug combinations for treatment of epidermoid carcinoma in order of objective response

Rank	Drugs	Response rate (%)	No. of patients	Randomized?	Ref. No.
1	5-Fluorouracil, procarbazine	55	11	Yes	19
2	Adriamycin, bleomycin, CCNU, mechlorethamine, vincristine	42	50	No	10
3	Cyclophosphamide, methotrexate, procarbazine, vincristine	28	46	Yes	5,6
4	CCNU, methotrexate, mechlorethamine	17	12	Yes	17
5	(a) Methotrexate, mechlorethamine	19	11	Yes	17
	(b) Adriamycin, CCNU, cyclophosphamide, procarbazine, hydroxyurea, methotrexate, vincristine	19	11	Yes	5
	(c) Adriamycin, CCNU, hydroxyurea	9	11	No	9
6	Mechlorethamine, CCNU	3	39	Yes	18

are available on the cardiotoxicity of adriamycin in patients with prior radiotherapy to the heart. Thus, special caution should be exercised in this group of patients.

The following tentative conclusions are drawn for the use of adriamycin in combination with other drugs for treatment of lung cancer:

1. Adriamycin is a useful component drug for the treatment of small-cell carcinoma.
2. Drug combinations including adriamycin are of interest for the treatment of epidermoid and large-cell carcinoma. Additional clinical data are needed to define the therapeutic value of these combinations when compared to alternative treatments.
3. Preliminary data give no evidence for activity of adriamycin in combination with other drugs against adenocarcinoma of the lung.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
BACON = bleomycin, adriamycin, CCNU, vincristine (Oncovin), and mechlorethamine (nitrogen mustard)
BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-
Bleomycin: NSC-125066
Busulfan: NSC-750; CAS reg. No. 55-98-1; 1,4-butanediol, dimethanesulfonate
Carbazilquinone: NSC-134679; CAS reg. No. 24279-91-2; *p*-benzoquinone, 2,5-bis(1-aziridinyl)-3-(2-hydroxy-1-methoxy)-6-methyl-, carbamate (ester)
CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
Colchicine: NSC-757; CAS reg. No. 7006-15-7
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Dehydroemetine: NSC-129414; emetine, 2,3-didehydro-, dihydrochloride, (+)-
Dibromodulcitol: NSC-104800; CAS reg. No. 10318-26-0; galactitol, 1,6-dibromo-1,6-dideoxy-
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8
Hexamethylmelamine: NSC-13875; CAS reg. No. 645-05-6
Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1
Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard; HN_2
6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-
1-Methyl-1-nitrosourea: NSC-23909; CAS reg. No. 684-93-5
Mitomycin C: NSC-26980; CAS reg. No. 50-07-7
NSC-45388: CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-
NSC-141540: 4′-demethylepipodophyllotoxin 9(4,6-*O*-ethylidene-β-D-glucopyranoside);epipodophyllotox. ethyl. gluc.
Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); Oncovin

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Combination Chemotherapy With Bleomycin (NSC-125066), Adriamycin (NSC-123127), CCNU (NSC-79037), Vincristine (NSC-67574), and Mechlorethamine (NSC-762) (BACON) in Squamous Cell Lung Cancer: Experience With 50 Patients[1,2]

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SUMMARY

Fifty patients with inoperable squamous cell carcinoma of the lung (38 with extensive disease and 12 with limited disease) were treated with BACON. Tumor regression greater than 50% was observed in 17 patients (45%) with extensive disease and in four patients (33%) with limited disease. Stabilization of disease for at least 8 weeks was seen in four patients with extensive and eight patients with limited disease, and this factor had the same prognostic importance as response with regard to survival. In extensive disease, responding and stable patients had a median survival time (MST) of 26 weeks from the start of therapy, while the MST for nonresponders was 9 weeks. The MST in patients with limited disease (all patients responding or stable) was 32 weeks. Analysis of survival in extensive disease by performance status showed improvement in each category over historical control results for placebo alone. BACON was better tolerated and probably more effective when CCNU was given every 8 weeks rather than every 4 weeks. Further studies are necessary to determine whether the results of this study can be reproduced in a comparative trial. If so, attention should be given to the treatment of less advanced squamous cell lung cancer with a similar regimen.

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Lung cancer is presently the most common malignancy in American men. The diagnosis is microscopically squamous cell (or epidermoid) in almost half the cases (1). Less than 25% of lung cancer patients are candidates for curative resection, and less than one third of this "favorable" subgroup survive 5 years (2). A representative analysis of survival was recently reported from the Veterans' Administration Lung Study Group (VALSG) for patients who are considered inoperable at the time of diagnosis (3). In that analysis, such patients were grouped by clinical extent of disease as well as by histologic type: patients with tumor clinically confined to one hemithorax (encompassable by a single radiotherapy port) had "limited" disease, and all others, including those who had received prior radiotherapy, had "extensive" disease. In 130 patients with limited squamous cell disease who received only supportive care, the median survival time (MST) was 15.7 weeks from the time the patient was determined inoperable. In 294 patients with extensive disease receiving supportive care only, the MST was 9.4 weeks.

Efforts to improve these results with radiotherapy and chemotherapy have so far met with very limited success. Patients with limited disease may have some improvement in survival from radiotherapy alone: in a large-scale randomized study of radiation therapy versus placebo, Roswit et al (4) found that treated patients survived about 20% longer than untreated control patients (MST, 20 versus 16 weeks respectively).

There is, to date, no conclusive evidence that any therapeutic modality improves survival in squamous cell lung cancer disseminated beyond the hemi-

[1]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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thorax at the time of diagnosis. Only one reported study (5), by the VALSG using mechlorethamine, purports to show a significant increase in survival: 26 versus 11 weeks for drug-treated patients and placebo-treated controls respectively. Unfortunately, close analysis of these results reveals that *(a)* patients with limited disease accounted for about one third of the population; *(b)* patients with "poorly differentiated" squamous cell cancer were not included in the survival analysis as squamous cell; and *(c)* patients with superior vena caval syndrome, or other problems felt to require immediate radiotherapy or chemotherapy, were not entered in the study at all. A recent large study by the Eastern Cooperative Oncology Group, reported by Edmonson et al (6), showed an MST of 11 weeks for patients with extensive squamous cell disease receiving mechlorethamine alone or in a two-drug combination with CCNU.

However, several drugs and drug combinations have been shown to cause objective tumor regression in patients with squamous cell lung cancer. Presumably, factors such as the rapid development of resistance, drug toxicity, and failure to favorably affect a *majority* of patients account for the lack of effect on increasing the MST. The reported response rates and survival data, when available, for selected single agents and combinations are shown in table 1.

Our own prior experience with combination attempts was in the COMB studies carried out from mid-1972 to mid-1973 at the M. D. Anderson Hospital in the Department of Developmental Therapeutics. These attempts were based on preclinical theoretical considerations and some encouraging preliminary results involving the two-drug combinations of methyl-CCNU plus cyclophosphamide and bleomycin plus vincristine. Vincristine was combined sequentially with bleomycin in the hope of potentiating the cytotoxic effect of the latter agent since *(a)* vincristine is stathmokinetic (7-10), producing a temporary but marked increase in mitotic index between 4 and 12 hours after its administration in man both in normal (7) and tumor tissues (7,9,10); *(b)* in cells not sensitive to bleomycin's cytotoxic effects, this increase in mitotic index reflects only temporary synchronization in the M phase (and, presumably, the G_2 phase), with subsequent escape into G_1 (7,8); and *(c)* both in vitro (11) and in vivo (12) studies had shown that cells in G_2 and M phases were preferentially sensitive to the cytotoxic effects of bleomycin. Methyl-CCNU and cyclophosphamide were combined

TABLE 1.—Response rates and survival data for single agents and combinations used in the treatment of squamous cell lung cancer

Drug(s)	Reference No.	No. of patients treated	Response (%)	Survival data
CCNU	18	29	41	*
Mechlorethamine	18	171	25	*
Methotrexate	18	140	25	*
Adriamycin	19	57	35	*
Cyclophosphamide	18	183	19	*
Bleomycin	18	75	9	*
Methotrexate, cyclophosphamide, vincristine, procarbazine		58	28	
Methotrexate, cyclophosphamide, vincristine, 5-FU, prednisone†		62	16	MST = 12 wks (all patients, extensive and limited disease), SWOG study
COMB				
1 and 2		22	45	MST = 9.5 wks (all patients), 12.5 wks (responders), 8.5 wks (nonresponders)
3		11	9	
1, 2, and 3		33	33	
BACON		50	42	MST = 20 wks (all patients) (see text of this paper)

*See text of indicated reference for survival data.
†Coltman C. Personal communication.

because of (*a*) broad-spectrum activity of each when used as single agents in human cancer, and (*b*) potent synergism of the combination in the Lewis lung carcinoma, a relatively low-growth-fraction animal tumor model (13). Pilot studies of combination therapy for squamous cell lung cancer were carried out at our institution: responses were produced with vincristine plus bleomycin in one of five patients (14) and with methyl-CCNU plus cyclophosphamide in one of six patients (15). No major overlap in toxicity between the two-drug combinations was observed. The initial four-drug regimen which resulted (COMB 1) used a dose of bleomycin totaling 60 mg/week for 6 weeks.

In COMB 2, the interval between courses of methyl-CCNU and cyclophosphamide was shortened from 6 to 4 weeks, and the dose of bleomycin was reduced to 30 mg/week, with a doubling in duration of therapy to achieve the same total dose (360 mg). As shown in table 1, the overall response rate of these two regimens was 45%. With COMB 3, the weekly dose of bleomycin was again cut in half for the same reason mentioned for COMB 2: to prolong the duration of responses by doubling the duration of administration of bleomycin. Unexpectedly, when the weekly dose was reduced to 15 mg (7.5 mg twice weekly), the response rate in squamous cell lung cancer fell to 9%, suggesting that a subeffective dose of bleomycin had been reached.

In spite of the encouraging response rate, the COMB studies failed to show an improvement in MST (overall MST of 9 weeks) for patients with squamous cell lung cancer, almost all of whom had extensive disease at the time of treatment. In addition, responders did not demonstrate significant improvement in survival over nonresponders (12 versus 8.5 weeks respectively), and it was found that prolonging the duration of vincristine and bleomycin beyond 6 weeks did not prolong the median response duration of 12 weeks.

COMB also produced a variety of disturbing toxic manifestations. Myelosuppression was marked, resulting in hospitalization for the administration of antibiotics in 20% of the patients and a 6% drug-related mortality rate on this basis. Probable clinically significant lung damage due to bleomycin occurred in 4% of the patients, contributing to patient death in each case. Thus, the overall incidence of drug-related mortality was 10%. In addition, almost all patients who received the twice-weekly combination of vincristine and bleomycin for more than 6 weeks had a "debilitation syndrome," characterized by weakness, anorexia, weight loss, and apathy. In general, this syndrome was reversible after vincristine and bleomycin were discontinued.

The BACON study was based on the COMB experience. The nonmyelosuppressive sequential vincristine-bleomycin combination was retained, but it was changed from twice weekly to once weekly, the total period of administration was limited to 6 weeks, and the weekly bleomycin dose was set at 30 mg. Mechlorethamine was substituted for the possibly less active cyclophosphamide. A nitrosourea was retained, CCNU being chosen because of the positive experience reported by others when it was used as a single agent (*see* table 1). A major change was the addition of adriamycin, a new agent with response rates in excess of 30% when given alone. The primary focus of the BACON study was in patients with primary squamous cell lung cancer; other programs were used for patients with other histologic types.

MATERIALS AND METHODS

All patients with a histologically confirmed diagnosis of unresectable squamous cell carcinoma were eligible provided they had not received previous chemotherapy with one of the study agents. Informed consent was obtained in every case. Specifically, patients were not excluded from the study because of compromised pulmonary function or evidence of organic heart disease.

Patients were defined as having limited disease when, on the basis of all clinical measurements, the tumor was confined to the hemithorax and ipsilateral mediastinal or supraclavicular nodes, and when prior radiotherapy had not been given. All others were classified as having extensive disease.

The modified Karnofsky scale as used by the VALSG (16) was employed to assess performance status (PS) at the start of the study in all patients.

The drug regimen used was as follows:

Day 1: CCNU was given in a dose of 65 mg/m^2 orally on an empty stomach. This was followed in 30 minutes by mechlorethamine (8 mg/m^2) and adriamycin (40 mg/m^2). Mechlorethamine was given by intravenous (iv) push slowly into the tubing of a freely flowing infusion. Adriamycin was given in the same way or as a rapid iv infusion in 50 ml of 5% dextrose in water.

Day 2: Vincristine was given as a rapid iv infusion or iv push in the morning in a dose of 0.75 mg for patients 50 years of age or over and 1 mg for those under 50. Bleomycin was given approximately 6 hours later in a dose of 30 mg as a rapid iv infusion.

CCNU was repeated in the same dose at 4-week intervals in the first 29 patients and at 8-week intervals in the last 21 patients. Mechlorethamine and adriamycin were repeated in the same dose at 4-week intervals. If the patient had a fall in absolute granulocyte count to less than 500 cells/mm^3, a reduction in platelet count to less than 50,000 platelets/mm^3, and/or morbidity related to leukopenia or thrombocytopenia after any course of myelosuppressive therapy, the doses of CCNU, adriamycin, and mechlorethamine were reduced by 25% for the next course. Vincristine and bleomycin were repeated weekly for a total of six doses and were then permanently discontinued.

A tumor regression was defined as a partial response if there was a 50% or greater decrease in the

TABLE 2.—Survival in extensive disease patients: BACON study vs VALSG historical control (placebo) study

PS	BACON pilot study		VALSG historical control (placebo)	
	No. of patients	Median survival (wks)	No. of patients	Median survival (wks)
1-4	11	9	73	2.5
5-7	26	20	141	10.5
8-10	1	35+	70	20

sum of the products of two perpendicular diameters of any measured lesions, without a simultaneous increase in the size of any lesion or the appearance of any new lesions. *Complete response* was defined as the disappearance of all clinical evidence of tumor. *Stable disease* was defined as a change of less than 50% (increase or decrease) in the size of measured tumor lesions, without the appearance of any new lesions, for at least 8 weeks. *Progressive disease* was defined as a greater than 50% increase in the size of any measured lesion and/or the development of new tumor lesions.

RESULTS

Response and Survival

Of 38 patients with extensive disease, 17 (45%) had greater than 50% regression of tumor. Four more were stable, for a total of 21 (55%) who appeared to have some antitumor effect. In 12 patients with limited disease, four (33%) had greater than 50% regression of tumor and eight were stable. Two patients with extensive disease had a complete response; in each case, this involved the disappearance of cervical nodes which were the only clinical evidence of disease.

The MST of all patients from the start of the study was 16 weeks for those with extensive disease and 20 weeks for the entire population. If survival from the start of therapy was examined by response category (fig 1), patients with extensive disease who had response or stabilization had an MST of 26 weeks (22 for responders and 30 for stable patients), while the MST of nonresponders was 9 weeks. This difference was significant with a P value of 0.002 by the standard two-tailed test. The patients with limited disease had an MST of 32 weeks. Survival was analyzed for patients with extensive disease by PS on the Karnofsky scale of 1-10. The results are shown in table 2 and are compared with the VALSG historical control experience for supportive care alone. For PS 1-4 the MST was 9 weeks and for PS 5-7 the MST was 20 weeks; these results compare favorably with the MSTs in the historical control experience. Only one patient was entered with extensive disease who had a PS of 8-10. The median duration of response was 16 weeks which did not differ according to CCNU schedule.

TABLE 3.—Survival according to type of CCNU schedule in BACON regimen

	Median survival (wks) with—	
	CCNU (q4wks)	CCNU (q8wks)
All patients	17.5	27+
Patients with extensive disease only	14.5	19
Patients in PS 5-7 only	20	27+

The responses were analyzed according to the CCNU schedule used: eight of 29 patients (28%) receiving the every-4-week schedule and 12 of 21 patients (62%) receiving the every-8-week schedule had a greater than 50% regression, with an additional eight patients receiving the every-4-week and four patients receiving the every-8-week schedule showing stable disease. The difference in response rates

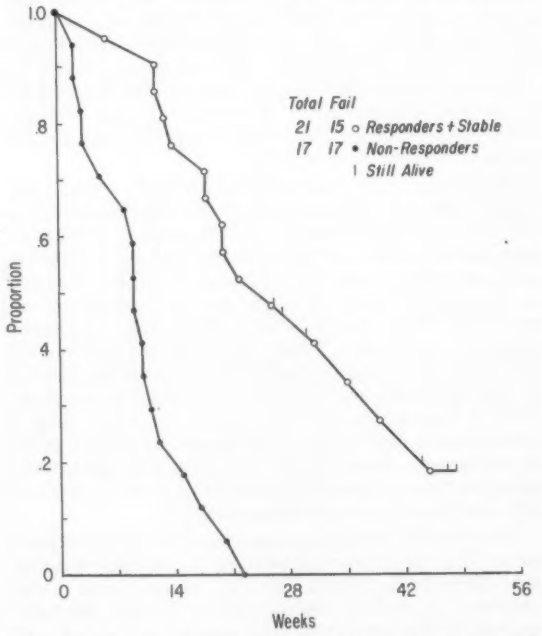

FIGURE 1.—Survival of patients with extensive disease receiving BACON.

TABLE 4.—Myelosuppressive toxicity according to type of CCNU schedule in BACON regimen

CCNU schedule	Median lowest value (cells × 10^3/mm^3)				No. of patients with—					
	Granulocytes		Platelets		<500 granulocytes/mm^3		<50,000 platelets/mm^3		<20,000 platelets/mm^3	
	1st course	Subsequent courses	1st course	Subsequent courses	1st course	Subsequent courses	1st course	Subsequent courses	1st course	Subsequent courses
q4wks	1.0	0.7	112	73	38	33	19	33	5	11
q8wks	1.0	1.0	No fall	128	22	25	11	16	0	6

TABLE 5.—Morbidity from myelosuppression according to type of CCNU schedule in BACON regimen

CCNU schedule	No. of patients	No. of patients with granulocyte toxicity and—			No. of patients with platelet toxicity and—	
		Leukopenia and fever	Proven infection	Drug-related death	Transfusion of platelets	Drug-related death
q4wks	29	5 (17%)	2 (7%)	1 (3.5%)	2 (7%)	1(3.5%)
q8wks	21	3 (14%)	1 (5%)	0	0	0
Both schedules	50	8 (16%)	3 (6%)	1 (2%)	2 (4%)	1 (2%)

was of borderline significance ($P = 0.1$). When survival was analyzed according to the CCNU schedule (table 3), the every-8-week schedule appeared to produce somewhat longer survivals, whether the comparison was for all patients, those with extensive disease only, or those in PS 5-7 only. This difference was not, however, significant for any category ($P>0.1$).

Toxicity

By far the most common important side effect of the BACON regimen was myelosuppression. Table 4 shows the comparative myelosuppressive effects for the 4-week schedule versus the 8-week schedule of CCNU and demonstrates the major reason for the change in schedule: there was a clear trend toward cumulative severity of thrombocytopenia with the more closely spaced nitrosourea schedule, with an increase in the incidence of severe thrombocytopenia (nadir < 20,000 platelets/mm^3) from the first courses to the subsequent courses (from 4.8% to 11%). When CCNU was given at 8-week intervals, the incidence of thrombocytopenia on subsequent courses appeared to be less (6%), and the same trend is apparent for lesser degrees of thrombocytopenia. Severe granulocytopenia (nadir < 500 absolute granulocytes/mm^3) did not appear to be cumulative on either schedule. Overall, 33 of 105 courses (31%) demonstrated this degree of granulocytopenia. The median number of days was 14 to the lowest granulocyte count and 16 to the lowest platelet count by either schedule.

Table 5 shows the morbidity related to myelosuppression for the two schedules of CCNU administration. Although there was no apparent difference between the two in terms of the incidence of hospitalization for leukopenia-related possible infection (16% of all patients), there was no significant morbidity related to thrombocytopenia on the more widely spaced schedule and no drug-related deaths secondary to myelosuppression, while there were two deaths among 29 patients (7%) receiving the earlier schedule.

Although pulmonary damage occurred, there was minimal morbidity from nonmyelosuppressive toxicity. Most patients had transient nausea and vomiting with adriamycin, mechlorethamine, and CCNU. None required hospitalization as a result. There were two cases of probable bleomycin-induced lung damage (4%) and one death from bleomycin. It may be relevant that the patient who died received simultaneous bleomycin and radiotherapy to the lungs (1500 rads in 1 week) for control of superior vena caval syndrome: a high incidence of bleomycin pulmonary toxicity has been observed in other patients receiving simultaneous bleomycin and pulmonary radiotherapy, and this will be reported further in a communication from Dr. Einhorn.

DISCUSSION

In squamous cell cancer of the lung, BACON has shown essentially the same encouraging response rate as COMB 1 and 2. However, a substantial number of patients receiving BACON also showed stabilization of disease as previously defined. This phenomenon was rarely observed with COMB. The result has been an apparent improvement in overall MST, from 9 weeks for COMB to 20 weeks for BACON. With BACON, the survival of responders and patients with stable disease is not significantly different; however, grouping them and comparing their survival to that of nonresponders indicates that the differences are highly significant (MST of 26 versus 9 weeks for responders and nonresponders respectively with extensive disease). Again, this is in contrast to the COMB experience, where responders showed no real difference from nonresponders in survival.

Thus, preliminary analysis indicates a prolongation of MST for patients with extensive (and limited) squamous cell lung cancer who are receiving BACON relative to that expected from historical control experience. These historical controls include the COMB studies at M. D. Anderson Hospital, in which the same physicians, the same response criteria, and a very similar patient population were involved. Patients with extensive disease achieving response or stabilization survived for a median of 4 months longer than nonresponders while receiving BACON. If these observations can be confirmed in larger-scale, cooperative studies, it would seem valid to conclude that an initial advance has been made. In particular, stronger emphasis might then be placed on the treatment of patients with minimal residual disease, in whom relatively more improvement in survival might be expected from a similar approach.

For such patients, the addition of radiotherapy may be especially important as an adjunct to effective chemotherapy. Immunotherapy may be of value in lung cancer patients with a limited tumor burden (16), and at least one recent report (17) suggests that it can greatly augment the results achieved with chemotherapy alone in melanoma patients with widely disseminated disease. Our current efforts are aimed at determining the efficacy of such combined modality approaches.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
BACON: combination of bleomycin, adriamycin, CCNU, vincristine (Oncovin), and mechlorethamine (nitrogen mustard)
Bleomycin: NSC-125066
CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
COMB: combination of cyclophosphamide, vincristine (Oncovin), methyl-CCNU, and bleomycin
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8
Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[p-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-
Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,-11,20-trione, 17,21-dihydroxy-
Procarbazine: NSC-77213; CAS reg. No. 491-88-3; p-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); Oncovin

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Use of Adriamycin (NSC-123127) in Hematologic Malignancies[1]

Peter H. Wiernik[2,3]

The use of adriamycin in the therapy of various hematologic malignancies has been carefully studied on an international scale by reliable investigators for more than half a decade. Several important conclusions about the proper role of this drug in the present-day treatment of hematologic malignancies can be drawn from this work, and I will close this presentation with my conclusions. First, I will present data collected from a variety of sources on the use of adriamycin alone and in combination with other antitumor agents.

Acute Lymphocytic Leukemia

Table 1 gives information on 148 previously treated children and adults with acute lymphocytic leukemia (ALL). The data were collected by the Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute (NCI) (1). Adriamycin was administered in various doses and schedules and the overall complete remission rate was 24%, with most investigators achieving rates well below that level. In these studies, remission was maintained by various methods, but remission duration was short—usually several months or less. It should be noted that most patients in the series reported by the Memorial Sloan-Kettering Cancer Center (MSKCC) had received daunorubicin sometime in the course of their disease (2).

Whitehouse et al (3) achieved a 35% complete response rate in 17 patients with previously treated ALL, including one patient who failed to respond to daunorubicin therapy.

Acute Nonlymphocytic Leukemia

Table 2 presents data on 47 previously treated children and adults with acute nonlymphocytic leukemia (ANLL) collected from several investigators who used a variety of doses and schedules of adriamycin. The overall complete remission rate was 23% (1). In the Roswell Park Memorial Institute (RPMI) experience (4) six patients had previously received daunorubicin and none were responsive to adriamycin. Whitehouse et al (3) recently reported on 13 patients previously treated with daunorubicin and cytosine arabinoside. None of the patients achieved a complete remission with adriamycin at a dose of 0.4 mg/kg daily for 2 or 3 days. Mathé et al (5) found four patients previously treated with daunorubicin to be refractory to adriamycin at a dose of 0.4 mg/kg daily for 4 days. Smith et al (6) have recently suggested that daunorubicin-resistant patients may respond to adriamycin at a much larger dose of 55-70 mg/m^2 daily for 1-3 days. It is not clear that their patients were in fact resistant to daunorubicin, and cytosine arabinoside was given in conjunction with adriamycin. Therefore, it is my judgement that the question of adriamycin-daunorubicin cross resistance in acute leukemia remains unsettled, although available data suggest that it is likely.

Currently, the Southwest Oncology Study Group (SWOG) is evaluating adriamycin at doses of 75 mg/m^2 every 3 weeks as a single agent in previously treated, relapsed adult patients with acute leukemia.[4] The large majority of the patients had ANLL. A total of 117 patients are currently evaluable and only 12% have achieved a complete remission. These data should be compared to four studies of daunorubicin alone in untreated patients with ANLL in which complete response rates of 44%-52% have been achieved (7-10).

A recent study at the M. D. Anderson Hospital (11) evaluated adriamycin, vincristine, cytosine arabinoside, and prednisone in previously untreated adults with acute leukemia. Complete remission was achieved in 83% of 46 patients with ANLL and in 69% of 13 patients with ALL. The median survival of all treated patients in that study is 33+ weeks. While these data represent some of the best results to date in the management of adult acute leukemia, it is still not known if they are superior to results obtained by others with daunorubicin and cytosine arabinoside in combination (12).

The above data indicate that current results with daunorubicin alone are superior to adriamycin alone in the treatment of ANLL. However, the data of Smith et al (6) suggest that when adriamycin is given in larger doses (60 mg/m^2 daily for 3 days), approximately the same dosage that achieved the best results with daunorubicin, the results with both anthracycline derivatives may be similar. Further work is necessary to determine the relative efficacy of these drugs and of rubidazone (13) in this disease.

Lymphomas

Table 3 gives response data on 147 patients with all types of lymphoma treated by several investigators using different adriamycin regimens. An overall

[1]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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[4]Wilson H. Personal communication, 1975.

TABLE 1.—Adriamycin activity in previously treated patients with ALL

Group	Total No. of patients	No. of— PR	No. of— CR	CR rate (%)
NCI, Milan	3	1	2	67
MSKCC	47	13	5	11
BCRC	5	0	0	0
RPMI	30	1	5	17
Mathé et al	63	7	24	38

TABLE 2.—Adriamycin activity in previously treated patients with ANLL

Group	Total No. of patients	No. of— PR	No. of— CR	CR rate (%)
MSKCC	6	1	2	33
RPMI	14	0	1	7
Mathé et al	27	3	8	30

TABLE 3.—Adriamycin activity in patients with previously treated advanced lymphoma

Group	Total No. of patients	No. of— PR	No. of— CR	CR rate (%)
NCI, Milan	63	18	7	11
MSKCC	22	7	—	—
SWCCSG*	47	21	—	—
BCRC	15	6	2	13

*SWCCSG = Southwest Cancer Chemotherapy Study Group.

41% response rate, with few complete responses, was obtained (1). Table 4 indicates that the drug as a single agent may be more active in histiocytic lymphoma than in the two other major classes of lymphoma.[5]

Recently, O'Bryan et al (14) reported a 43% response rate in 48 patients with a variety of lymphomas and Cimo et al (15) obtained a 36% response rate in 14 patients with previously treated far-advanced lymphoma. Gottlieb et al (16) reported on 18 patients with previously treated lymphoma who received adriamycin at a dose of 60-75 mg/m^2 every 3 weeks. Five complete and four partial responses (50%) were obtained and the median duration of remission was 7+ months. Patients with lymphocytic and histiocytic lymphoma responded equally well. Similarly, no advantage for nodular or diffuse histology was observed.

The best therapeutic results with adriamycin for lymphoma have been obtained by use of the drug in combination with other active agents. The SWOG is comparing a combination of cyclophosphamide, adriamycin, vincristine, and prednisone against the same combination minus cyclophosphamide in non-Hodgkin's lymphoma.[6] Complete remission rates of 35%-73% are being obtained in the various histologic subtypes of lymphoma with only minor differences between the two arms of the study to date.

Other examples of successful adriamycin combination therapy for malignant lymphoma include the work of Monfardini et al (17) in non-Hodgkin's lymphoma and of Bonadonna et al (18) in advanced Hodgkin's disease. Monfardini (17) used the high-dose combination of adriamycin, bleomycin, and prednisone (ABP) shown below:

Adriamycin (75 mg/m^2) intravenously (iv), Day 1
Bleomycin (15 mg/m^2) intramuscularly (im), Days 1 and 8
Prednisone (100 mg/m^2) orally, Days 1-5

This regimen was repeated every 3 weeks. This combination of agents was compared to cyclophosphamide, vincristine, and prednisone (CVP) in patients with untreated non-Hodgkin's lymphoma and was found to be superior for remission induction.

TABLE 4.—Responses to adriamycin in lymphoma subtypes

Lymphoma	No. of patients treated*	Response rate (%)
Hodgkin's disease	67	33
Histiocytic lymphoma	50	48
Lymphocytic lymphoma	44	32

*Most patients had advanced, previously treated disease.

[5]Carter SK. Personal communication, Mar 16, 1973.
[6]McKelvey E. Personal communication, Jan 1975.

TABLE 5.—CVP vs ABP for untreated non-Hodgkin's lymphoma

Regimen	Lymphocytic lymphoma		Histiocytic lymphoma	
	CR + PR (%)	Median response duration (mos)	CR + PR (%)	Median response duration (mos)
CVP	71	4.0	73	6.0
ABP	87	3.5	89	2.5

However, remission duration with ABP was disturbingly short. Cross resistance did not occur with these regimens (table 5).

Bonadonna (18) used the combination of adriamycin, bleomycin, vinblastine, and NSC-45388 (ABVD) for stage III and IV Hodgkin's disease shown below:

Adriamycin (25 mg/m^2) iv, Days 1 and 14
Bleomycin (10 mg/m^2) im, Days 1 and 14
Vinblastine (6 mg/m^2) iv, Days 1 and 14
NSC-45388 (150 mg/m^2) iv, Days 1-5

The regimen was repeated six times. Results were identical to those obtained with MOPP in a randomized study. Again, cross resistance was not demonstrated in patients who relapsed or in those with progressive disease (table 6).

Three studies are currently in progress at the Baltimore Cancer Research Center (BCRC) that may be of interest. The first is a monthly combination of CCNU, bleomycin, adriamycin, and streptozotocin (SCAB) for advanced, refractory Hodgkin's disease (table 7). Six patients who were failures on MOPP therapy are currently evaluable. Three have obtained complete remissions, one has obtained a partial remission, and two are treatment failures. The complete remissions are of 3, 5+, and 6+ months' duration.

The second BCRC study is a combination of high-dose cyclophosphamide, adriamycin, vincristine, and prednisone (CAVP) for stage III and IV diffuse histiocytic lymphoma (table 8). Patients are treated in a laminar air-flow (LAF) room and receive oral non-absorbable antibiotics as well. The course of chemotherapy is repeated as soon as recovery from myelosuppression allows, usually 2-3 weeks. Thus far, only two patients have been entered in this study and both have achieved complete remission.

In the third BCRC study adriamycin is given at a dose of 60 mg/m^2 every 3 weeks as a single agent to patients with advanced, previously treated mycosis fungoides. Thus far, eight patients have been treated and six have had good partial responses of 1+ to 12+ months' duration.

These studies in lymphoma indicate that while adriamycin alone is highly active in the lymphomas, response rates and remission duration are significantly improved when this drug is used in combination with other active agents.

Multiple Myeloma

O'Bryan et al (14) obtained two significant responses in 21 patients with advanced myeloma unresponsive to alkylating agents. Recently, Alberts et al (19) treated nine myeloma patients who had failed to respond to intermittent therapy consisting of melphalan and prednisone with adriamycin given at a dose of 25-45 mg/m^2 every 1-3 weeks. Three patients had a favorable response. These data suggest that it may be profitable to study adriamycin in combination with melphalan or cyclophosphamide in patients with less advanced myeloma.

TABLE 6.—ABVD vs MOPP for stage IIIB, III$_s$, and IV Hodgkin's disease

Regimen	No. of patients treated	CR rate (%)
MOPP	22	72
ABVD	17	70

TABLE 7.—SCAB for advanced refractory Hodgkin's disease (BCRC protocol No. 7402)

Drug	Monthly dose schedule	Days of therapy							
		1	2	3	4	5	6	7	8
Streptozotocin	0.5 g/m^2 iv	×	×	×	×	×			
CCNU	100 mg/m^2 orally	×							
Adriamycin	45 mg/m^2 iv	×							
Bleomycin	15 mg/m^2 im	×							×

TABLE 8.—CAVP for stage III and IV diffuse histiocytic lymphoma (BCRC protocol No. 7309)

Drug*	Dose schedule (q2-3wks)	Days of therapy									
		1	2	3	4	5	6	7	8	9	10
Cyclophosphamide	1.5 g/m² iv	×									
Adriamycin	60 mg/m² iv	×									
Vincristine	2 mg iv	×									
Prednisone	40 mg/m² orally	×	×	×	×	×	×	×	×	×	×

* Patients use the laminar air flow room and receive oral gentamycin, nystatin, and vancomycin during therapy.

Recommendations for the Use of Adriamycin in Hematologic Malignancies

The following recommendations are offered as a guide to the use of this new agent by practicing oncologists. Deviations from these suggestions may be entirely appropriate in a research setting:

1. Indications for adriamycin alone in hematologic malignancies are few, but probably include mycosis fungoides with extracutaneous involvement, multiple myeloma unresponsive to alkylating agents, and perhaps, untreated myeloma with severe renal failure.
2. Inappropriate uses of adriamycin alone in hematologic malignancies include use in ALL and probably ANLL. In addition, non-Hodgkin's and Hodgkin's lymphoma are not appropriate indications for adriamycin alone.
3. Inappropriate uses of adriamycin alone or in combination in hematologic malignancies include induction therapy of untreated childhood ALL, adjuvant therapy with radiation in Hodgkin's disease confined to lymph nodes, initial therapy of nodular poorly differentiated lymphocytic lymphoma, and maintenance therapy in any disease.
4. Hematologic malignancies in which the role of adriamycin has not been studied include chronic lymphocytic leukemia, chronic myelocytic leukemia, and Waldenström's neuroglobulinemia. Therefore, the role, if any, of adriamycin in the therapy of these diseases cannot be ascertained at present.
5. When adriamycin is used for the treatment of lymphoma it should be combined with other active agents for maximal effect. However, in previously untreated patients, other less toxic drugs in combination may be as effective as combinations including adriamycin.

In summary, adriamycin is a new antitumor agent that has shown significant activity in every hematologic malignancy in which it has been studied. Of particular interest to the practicing oncologist is the usefulness of this drug in combination with other active drugs for the treatment of advanced Hodgkin's and non-Hodgkin's lymphoma. The lack of cross resistance between chemotherapy regimens containing adriamycin and more standard drug treatments allows for more adequate treatment of lymphoma patients who fail to respond to initial standard approaches. Studies in progress throughout the world should ultimately define the role of adriamycin in the primary therapy of patients with hematologic malignancies.

LIST OF COMPOUNDS

ABP = adriamycin, bleomycin, and prednisone
ABVD = adriamycin, bleomycin, vincristine, and DTIC
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
Bleomycin: NSC-125066
CAVP = cyclophosphamide, adriamycin, vincristine, and prednisone
CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
CVP = cyclophosphamide, vincristine, and prednisone
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6
DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-
Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride
Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-
MOPP = mechlorethamine, vincristine, prednisone, and procarbazine
Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-
Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride
Rubidazone: NSC-164011; CAS reg. No. 36508-71-1; daunorubicin, benzoylhydrazone, monohydrochloride
SCAB = streptozotocin, CCNU, adriamycin, and bleomycin
Streptozotocin: NSC-85998
Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Adriamycin (NSC-123127) in the Treatment of Lymphomas: Southwest Oncology Group Studies[1]

Charles A. Coltman, Jr. [2,3]

SUMMARY

The Southwest Oncology Group has used adriamycin alone and in combination with other agents in the treatment of Hodgkin's disease and nonHodgkin's lymphoma. When a single 75-mg/m² dose was given every 3 weeks, the complete plus partial response rate was 39% in patients with Hodgkin's disease and 44% in those with nonHodgkin's lymphoma. These patients had had extensive prior chemotherapy. An adriamycin dose-response study subsequently compared the single dose of 75 mg/m² with that of 45 mg/m² given every 3 weeks. There is some evidence of a dose-response relationship in nonHodgkin's lymphoma (45% versus 29% response rate). Adriamycin was added to cyclophosphamide, vincristine, and prednisone (CHOP) and was compared with adriamycin, vincristine, and prednisone (HOP) in the treatment of patients with advanced nonHodgkin's lymphoma. The complete response rate was 55% for CHOP and 50% for HOP. The toxicity of these two regimens was marked. The durations of complete response were no different in the two limbs, and the Kaplan and Meier estimate of the 2-year disease-free interval is 75% for CHOP and 60% for HOP. A pilot study was done to test the feasibility of adding adriamycin to the best prior regimen used in advanced Hodgkin's disease, which was mechlorethamine, vincristine, prednisone, and procarbazine (MOPP) plus low-dose bleomycin. Two schedules were tested: *(a)* 30 mg/m² of adriamycin was given in place of mechlorethamine on Day 8 of each of ten cycles of MOPP plus low-dose bleomycin; and *(b)* 15 mg/m² of adriamycin was added on Days 1 and 8 of each of ten MOPP plus low-dose bleomycin cycles with an attenuated dose (3 mg/m²) of mechlorethamine on Days 1 and 8. These well-tolerated regimens are now being compared to MOPP plus low-dose bleomycin in advanced Hodgkin's disease in a group-wide study.

[Cancer Chemother Rep Part 3, vol 6:375-380, 1975]

The new anthracycline antibiotic, adriamycin, has been used both alone (1-4) and in combination (4,5) in patients with lymphoma of various types. The Southwest Oncology Group (SWOG) has been involved in studies testing the value of adriamycin alone and in combination in the management of Hodgkin's disease and nonHodgkin's lymphoma. This brief review covers the experience with adriamycin alone, given in a single dose every 3 weeks, and its use in a dose-response evaluation. Also covered is a preliminary report on a major SWOG study in which adriamycin was used in combination with cyclophosphamide, vincristine, and prednisone (CHOP) in the management of disseminated nonHodgkin's lymphoma. Finally, a pilot study is presented in which adriamycin was added in two different schedules to mechlorethamine, vincristine, procarbazine, prednisone, and bleomycin at a low dose level (MOPP + ldB) in the treatment of advanced Hodgkin's disease.

ADRIAMYCIN ALONE

The initial SWOG study with adriamycin alone (3) used a dose of 75 mg/m² every 3 weeks for "good-risk" patients. A dose of 60 mg/m² was used for "poor-risk" patients. A response of a 50% or greater reduction in the sum of the products of the largest diameter and its perpendicular of all measurable disease for a minimum period of 4 weeks was noted in seven of 18 (39%) patients with Hodgkin's disease and in seven of 16 (44%) patients with histiocytic lymphoma.

After evaluating adriamycin alone, an attempt was made to determine if a lower dose of adriamycin (45 mg/m², every 3 weeks) was as effective as the 75-mg/m² dose used in the original study (3). Patients were randomly allocated to one of two treatment regimens: high-dose (75 mg/m²) and low-dose (45 mg/m²). A total of 15 patients with Hodgkin's disease

[1]Adriamycin was supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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and 44 patients with nonHodgkin's disease were entered into the study (table 1).[4] The number of patients with Hodgkin's disease was small but there was evidence of a dose-response relationship in patients with nonHodgkin's lymphoma with respect to both response rate (45% versus 29%) and remission duration (median of 6.4 months versus 1.9+ months). These data are compatible with the hypothesis that adriamycin is less effective in the low-dose (45 mg/m^2) regimen than it is in the high-dose (75 mg/m^2) regimen in patients with nonHodgkin's lymphoma.

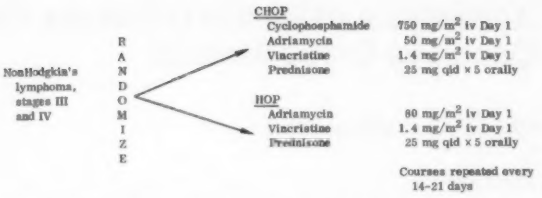
NonHodgkin's lymphoma, stages III and IV
RANDOMIZE
CHOP
Cyclophosphamide 750 mg/m2 iv Day 1
Adriamycin 50 mg/m2 iv Day 1
Vincristine 1.4 mg/m2 iv Day 1
Prednisone 25 mg qid x 5 orally
HOP
Adriamycin 80 mg/m2 iv Day 1
Vincristine 1.4 mg/m2 iv Day 1
Prednisone 25 mg qid x 5 orally
Courses repeated every 14-21 days

FIGURE 1.—Remission-induction treatment schemes for CHOP versus HOP in patients with stages III and IV nonHodgkin's lymphoma.

TABLE 1.—Adriamycin dose-response study*

	Adriamycin dose (mg/m^2)	No. of patients responding/ No. treated†	Response rate (%)	Median duration of response (mos)
Hodgkin's disease	75	4/10	40	5.8
	45	2/5	40	1.5+
NonHodgkin's lymphoma	75	9/20	45	6.4
	45	7/24	29	1.9+

*See footnote 4.
†Complete plus partial response.

ADRIAMYCIN IN COMBINATION

NonHodgkin's Lymphoma

The SWOG has conducted a series of studies in patients with nonHodgkin's lymphoma using cyclophosphamide, vincristine, and prednisone (COP) (6). The most recent study, initiated in April 1972, was based on a pilot study conducted at the M.D. Anderson Hospital and Tumor Institute (Houston, Texas) (4) in which CHOP was used. Of nine patients in the pilot study who had received little prior chemotherapy, seven achieved complete responses and two achieved partial responses. All patients tolerated the therapy without difficulty.

Prompted by the impressive response rate, the SWOG undertook a study which compared CHOP with adriamycin, vincristine, and prednisone (HOP) in patients with stages III and IV nonHodgkin's lymphoma (fig 1).[5] Both cyclophosphamide and adriamycin doses were reduced when combined in the CHOP limb (from 1000 to 750 mg/m^2, and from 80 to 50 mg/m^2, respectively), because both agents are myelosuppressive. Each regimen was repeated every 14-21 days as tolerated. Patients were treated until a complete response was attained, after which they received three consolidation courses prior to randomization to maintenance therapy. No patient received a dose of adriamycin greater than 550 mg/m^2.

Table 2 shows the patient entries into the remission-induction limbs of this study as of September 1974. The partially evaluable patients in the CHOP (33 patients) and HOP (30 patients) limbs include patients who had an inadequate trial due to early death, toxicity, being lost to followup, or refusing further therapy. All of the partially evaluable patients were included in the denominator of the calculations for response rates and remission durations.

TABLE 2.—Information on patients entered into remission-induction study: CHOP versus HOP*

	No. of patients	
	CHOP	HOP
Fully evaluable	179	177
Partially evaluable		
Early death	14	8
Inadequate trial, toxicity	8	12
Lost to followup	4	6
Refused further therapy	2	4
Other	5	0
Total	33	30
Total No. evaluable	212	207
Too early to evaluate	67	66
Not evaluable		
Major protocol violation	1	2
Insufficient data	3	3
Total	4	5
No. of patients included in this analysis	283	278

*SWOG protocol study No. 7204; preliminary analysis, Sept 1974.

[4]O'Bryan RM, Coordinator. SWOG study No. 7206, personal communication.

[5]McKelvey EM, Coordinator. SWOG study Nos. 7204 and 7205, CHOP/HOP, personal communication.

Patients in the two limbs of the study were comparable in the pretreatment characteristics of age, sex, pathologic stage, histologic classification, and extent of prior therapy. Results of therapy in the evaluable and partially evaluable patients who *completed* the remission-induction phase of this protocol are listed in table 3. There was a complete response rate of 55% in the CHOP limb and 50% in the HOP limb. There is no statistically significant difference in the complete response rates of these two induction regimens when one considers either the evaluable or adequate-trial groups. A preliminary estimate of response rate with respect to histologic categories shows no significant difference in response rate in the two treatment groups except for the well-differentiated lymphocytic categories. In these categories there was a 73% (16 of 22 patients) complete response rate with CHOP as compared to a 41% rate (seven of 17) with HOP in the nodular category, and a 55% (12 of 22) complete response rate with CHOP as compared to a 35% rate (nine of 26) with HOP in the diffuse well-differentiated lymphocytic category. This finding suggests that cyclophosphamide has made a contribution to the complete response rate in that cell type.

Table 4 tabulates the most severe types of toxicity and the degree of toxicity in each patient. Table 5 tabulates the worst degree of toxicity in each patient. Leukopenia, thrombocytopenia, and infection were severe in both limbs of the study. Although there was no significant difference in the types or degree of toxicity between CHOP and HOP, 11 patients died during the CHOP regimen and seven during the HOP regimen. One patient in each limb died of irreversible renal failure after the development of uric acid nephropathy. Congestive heart failure due to adriamycin treatment was not seen in this study. Both regimens were more toxic than the prior experience with COP alone (6).

The median duration of complete remission had not been reached in either of the limbs of the study as of September 1974. The Kaplan and Meier estimate of the percentage of patients who are relapse-free at 2 years is 75% for CHOP and 60% for HOP. The differences between remission-duration curves are not statistically significant ($P = 0.17$).

TABLE 3.—Results of CHOP versus HOP therapy in patients who completed the remission-induction phase*

	CHOP	HOP
Total No. of evaluable patients	164	166
Total No. of adequate-trial patients	142	145
No. of patients with—		
Complete response (CR)	91	83
Partial response (PR)	32	38
	123	121
Response rate (evaluable patients)		
CR	55%	50%
CR + PR	75%	73%
Response rate (adequate-trial patients)		
CR	64%	57%
CR + PR	86%	83%

*Preliminary analysis, Sept 1974.

After complete remission and consolidation, patients were randomly assigned to a remission-maintenance regimen of either COP or vincristine, cytosine arabinoside, and prednisone given at monthly intervals for 18 months. The remission duration and overall survival data are as yet too preliminary to report.

Adriamycin added to a modified COP regimen (6) produces an approximately 50% complete response rate with no significant difference when cyclophosphamide is deleted and the adriamycin dose is increased. Cyclophosphamide appears to have improved the complete response rate only in the well-differentiated lymphocytic category. The early remission durations were quite long and were better than those previously achieved by the SWOG. These are toxic regimens and should be used with care in the elderly patient.

Hodgkin's Disease

Since 1966, the SWOG has been involved in a series of studies testing the effectiveness of various combination chemotherapy regimens in the management of advanced Hodgkin's disease (table 6). Between 1968 and 1971, 384 patients with advanced Hodgkin's disease were treated with MOPP, as outlined by Frei et al (7), resulting in a 63.5% complete response rate. In 1971, the SWOG began a study which compared MOPP alone with MOPP plus two different dose levels of bleomycin. Bleomycin was added to MOPP at a dose level of 2 units/m^2 on Days 1 and 8 of the first six cycles on the low-dose schedule (MOPP + ldB) and 10 units/m^2 on Days 1 and 8 of the second through the sixth cycles on the high-dose schedule (MOPP + hdB). A test dose of 5 units was given on Days 1 and 8 of the first course in the high-dose regimen to avoid the anaphylactic-like reactions noted with bleomycin in lymphoma patients.[6] The MOPP + hdB limb was closed early because irreversible myelosuppression developed in three patients. The MOPP-alone limb was discontinued at the same time because the complete response rate (67%) was comparable to that of the prior SWOG experience with MOPP alone (63.5%) and it was not likely that it could ever be better than the MOPP + ldB schedule. The 85% complete response rate includes all fully evaluable patients as well as those who had inadequate trials.

Bonadonna et al (8) have employed mechlorethamine, vincristine, prednisone, bleomycin, and adriamycin in patients with advanced Hodgkin's disease. Of 32 patients, 72% achieved a complete response after receiving six cycles of therapy. Based on the preliminary SWOG experience with MOPP + ldB, Jones[7]

[6]Agre K. Beloxane Product Monograph, Bristol-Myers Company, 1972.

[7]Jones SE, Coordinator. Personal communication.

TABLE 4.—No. of patients with toxicity and degree of toxicity in the CHOP versus HOP regimens*

	CHOP						HOP					
Toxic effect	None	Mild	Moderate	Severe	Life threatening	Fatal	None	Mild	Moderate	Severe	Life threatening	Fatal
Leukopenia	41	15	52	62	32	5	42	21	45	64	32	3
Thrombocytopenia	171	15	5	7	7	2	172	6	15	5	8	1
Nausea	174	12	15	6	0	0	191	5	5	2	0	0
Mucositis	196	1	5	5	0	0	187	6	5	8	1	0
Neurotoxicity	152	26	22	5	2	0	154	17	24	12	0	0
Alopecia	157	0	9	41	0	0	155	2	12	38	0	0
Cardiovascular	201	5	0	1	0	0	145	9	3	0	0	0
Infection	191	1	3	3	0	9	200	0	1	1	1	4

*SWOG protocol study No. 7204; preliminary analysis, Sept 1974.

TABLE 5.—Worst degree of toxicity in each patient in the CHOP versus HOP regimens*

Regimen	Degree				
	None or mild	Moderate	Severe	Life threatening	Fatal
CHOP	37	40	87	32	11
HOP	36	41	90	33	7

*SWOG protocol study No. 7204; preliminary analysis, Sept 1974.

TABLE 6.—SWOG studies of various combination chemotherapy regimens in the treatment of advanced Hodgkin's disease

Years	Reference No.	Study	Total No. of patients	Complete response rate (%)
1966-1968	6	COP No. 1	107	36
1968-1969	7	MOPP Nos. 1 and 2	178	66
1969-1971	9	MOPP No. 3	206	62
1971-1974	10	MOPP alone	46	67
		MOPP + hdB	43	72
		MOPP + ldB	75	85

devised a program which adds adriamycin in two different schedules (table 7). Adriamycin is added at a dose level of 30 mg/m^2 on Day 8 in place of mechlorethamine in the first schedule (MOP-BAP No. 1). Adriamycin is added at a dose level of 15 mg/m^2 on Days 1 and 8 with an attenuated dose (3 mg/m^2) of mechlorethamine in the second schedule (MOP-BAP No. 2). A total of 13 patients with advanced Hodgkin's disease, four from the University of Arizona[8] and nine from Wilford Hall USAF Medical Center (WHMC), have been treated in one or both of the limbs of this pilot study. The program calls for ten cycles of therapy followed by unmaintained remission in those patients whose complete response is clearly documented by systematic restaging. Seven patients are still in the treatment program, having received from two to eight cycles of MOP-BAP No. 1 and/or MOP-BAP No. 2. Three patients have completed all ten cycles of therapy and remain in complete remission at 6+, 5+, and 3+ months, respectively. Three patients are partially evaluable (one patient in each category: early death, lost to follow-up, and refused further therapy). Ten patients had pathologic stage IVB and three had stage IVA Hodgkin's disease. Five patients had extensive prior chemotherapy, three had extensive prior radiotherapy, and five had no prior radiotherapy.

[8]One patient was found to have histiocytic lymphoma after being reviewed by Dr. James J. Butler of the Pathology Panel and Repository Center for Lymphoma Clinical Trials.

TABLE 7.—Adriamycin used in two different schedules

Drug and route of administration	Treatment (mg/m^2) on Days—			
	1	2-7	8	9-12
MOP-BAP No. 1				
Mechlorethamine, iv	6			
Vincristine, iv	1.4		1.4	
Procarbazine, orally		100		100
Prednisone, orally*		40		40
Bleomycin, iv	2		2	
Adriamycin, iv			30	
MOP-BAP No. 2				
Mechlorethamine, iv	3		3	
Vincristine, iv	1.4		1.4	
Procarbazine, orally		50		50
Prednisone, orally*		40		40
Bleomycin, iv	2		2	
Adriamycin, iv	15		15	

*Cycles 1, 4, 7, 10, etc.

TABLE 8.—Median time between courses and median blood cell count nadirs for each course of MOP-BAP Nos. 1 and 2*

	Course No.—									
	1	2	3	4	5	6	7	8	9	10
Total No. of courses administered	9	8	7	6	6	5	4	4	3	3
Median time between courses (days)	28	29	28	35	28	28	28	28	28	
Granulocyte count nadir ($\times 10^3$ cells/μl)	1.6	1.9	1.6	1.5	1.4	2.4	1.7	1.5	1.7	
Platelet count nadir ($\times 10^3$ platelets/μl)	386	432	292	200	232	270	339	280	248	

*Based on the 55 courses given to nine patients at WHMC.

Patients initially received MOP-BAP No. 1; later, several patients were switched to MOP-BAP No. 2 and additional patients received only MOP-BAP No. 2 alone. Seven patients (four from Arizona and three from WHMC) received a total of 37 cycles of MOP-BAP No. 1 and eight patients (two from Arizona and six from WHMC) received a total of 35 cycles of MOP-BAP No. 2. The toxicities in both limbs of the pilot study included alopecia, nausea and vomiting, and tolerable myelosuppression. Table 8 shows the median time between courses and the median granulocyte and platelet nadirs during the ten cycles (nine patients from WHMC). Myelosuppression was minimal and patients' acceptance of mild nausea and vomiting was higher than in prior MOPP studies. Because of the high level of activity and acceptable level of toxicity in these two regimens, the SWOG designed a group-wide protocol[9] to test prospectively the best limb of our previous study (MOPP + ldB) with MOP-BAP No. 1 and MOP-BAP No. 2 in a randomized study. Patients will receive ten cycles of therapy and those who achieve complete responses, as documented by systematic restaging, will be followed in unmaintained remission.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8; hydroxydaunomycin

Bleomycin: NSC-125066

CHOP = adriamycin, cyclophosphamide, vincristine, and prednisone

COP = cyclophosphamide, vincristine, and prednisone

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

HOP = adriamycin, vincristine, and prednisone

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard

MOP-BAP Nos. 1 and 2 = mechlorethamine, vincristine, procarbazine, prednisone, bleomycin, and adriamycin

MOPP = mechlorethamine, vincristine, prednisone, and procarbazine

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21 -dihydroxy-

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); Oncovin

[9]Jones SE, Coordinator. SWOG study No. 7406, MOPP No. 5, personal communication.

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Combination Usage of Adriamycin (NSC-123127) in Malignant Lymphomas[1]

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SUMMARY

The results obtained in advanced malignant lymphomas with three new drug combinations including adriamycin are reported in this paper. In patients with Hodgkin's disease, the quadruple combination of adriamycin, bleomycin, vinblastine, and NSC-45388 (ABVD) in a controlled trial produced a percent of complete remission and a median duration of response comparable to those obtained with mechlorethamine, vincristine (Oncovin), prednisone, and procarbazine (MOPP). Similar results were observed in the group of patients with nonHodgkin's lymphomas where adriamycin, bleomycin, and prednisone (ABP) were randomly tested against cyclophosphamide, vincristine, and prednisone (CVP). Initial evidence shows a lack of cross resistance between ABVD and MOPP as well as between ABP and CVP. These data favor the design of new combinations and new strategic approaches for the best management of patients with advanced malignant lymphomas. Combinations that include adriamycin and bleomycin may, however, produce severe toxicity (cardiomyopathy, lung fibrosis) when these drugs are used as long-term therapy.

[Cancer Chemother Rep Part 3, vol 6:381-388, 1975]

A number of clinical studies carried out in several institutions over the past 5 years have confirmed the usefulness of adriamycin as a single agent in the treatment of Hodgkin's disease and nonHodgkin's lymphomas (1-7). Among the different histopathologic types, histiocytic lymphomas proved to be the most responsive to this new agent (2,5). Most patients had disseminated disease refractory to radiotherapy, or conventional agents, or both. Therefore, as in other advanced neoplasias, available data might considerably underestimate the effectiveness of adriamycin especially in terms of incidence and duration of complete remission.

Table 1 summarizes the results obtained during phase II studies in our Institute. The table includes for comparison the therapeutic results obtained with another antitumor antibiotic, bleomycin, with which a phase II study was carried out during approximately the esist- ɔeriod of time. The data show that both drugs appear effective in advanced lymphomas, although the median duration of response was short. Despite the limitations of retrospective comparisons, there seems to be no statistical difference between the response rates obtained with adriamycin and bleomycin.

On the basis of these findings, a cyclic five-drug combination was designed for patients with Hodgkin's and nonHodgkin's lymphomas in 1970 (8). The drug schedule was similar to that employed with mechlorethamine, vincristine (Oncovin), prednisone, and procarbazine (MOPP), although procarbazine was replaced with adriamycin and bleomycin. More recently, when it became clear that all MOPP-modified regimens failed to yield better results in Hodgkin's disease (incidence and duration of complete remission) than MOPP (9,10), adriamycin was included in a new four-drug combination which was tested against MOPP. The intent of this new study was to find an effective noncross-resistant regimen which could be employed either in patients who failed to respond to treatment with MOPP or in sequence with the MOPP regimen. The same strategic concept was applied in patients with nonHodgkin's lymphomas using a new three-drug regimen.

This paper collects and updates most of the data derived from our trials performed in all types of advanced malignant lymphomas during the past 5 years.

PATIENTS AND METHODS

Histology and Staging

All patients entered into the studies between 1970 and 1974 had histologically proven diagnoses of malignant lymphoma. In patients biopsied elsewhere,

[1]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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TABLE 1.—Comparative responses to adriamycin and bleomycin in advanced refractory malignant lymphomas

Histology	No. of evaluable patients	% CR + PR	No. of patients with CR	Response rate (%)	Median duration (mos)
Adriamycin					
Hodgkin's disease	27	37	1	78	4.5
Histiocytic lymphoma	19	79	2	89	2.5
Lymphocytic lymphoma	13	46	1	84	2.5
Totals	59*	53	4	83	
Bleomycin					
Hodgkin's disease	21	57	2	81	2
Histiocytic lymphoma	11	27	0	73	1+
Lymphocytic lymphoma	6	66	1	66	2
Totals	38*	50	3	76	

*No. of patients previously treated with radiotherapy and/or chemotherapy: adriamycin = 45/59; bleomycin = 38/38.

slides, or paraffin blocks, or both were reviewed by members of the Division of Pathology of the Institute. The histopathologic classifications employed were those of Lukes and Butler (11) for patients with Hodgkin's disease and of Rappaport et al (12) for patients with nonHodgkin's lymphomas.

Patients were staged or restaged according to the criteria recommended at Ann Arbor in 1971 (13). Whenever possible, a pathologic staging with either peritoneoscopy, or laparotomy, or both was carried out. Clinical staging included a unilateral marrow biopsy with Jamshidi needle, especially in patients who were entered within the past 2 years.

Drug Regimens

Since 1970, adriamycin has been included in three protocols of combination chemotherapy for patients with Hodgkin's disease and nonHodgkin's lymphomas. Before being entered into the study, all patients were in fairly good condition (performance status greater than 40). With the exception of a small fraction of those receiving the five-drug regimen, patients had not received previous chemotherapy. The studies were designed to avoid administering a dose level of adriamycin higher than 600 mg/m^2 during the period of induction therapy.

Figure 1 reports the three-drug combinations employed. The details of the studies have been reported elsewhere (8,10,14,15). From the first combination of mechlorethamine, vincristine, adriamycin, bleomycin, and prednisone (MABOP) employed for all types of malignant lymphoma, two new drug regimens were derived: (*a*) adriamycin, bleomycin, vinblastine, and NSC-45388 (ABVD) for Hodgkin's disease; and (*b*) adriamycin, bleomycin, and prednisone (ABP) for

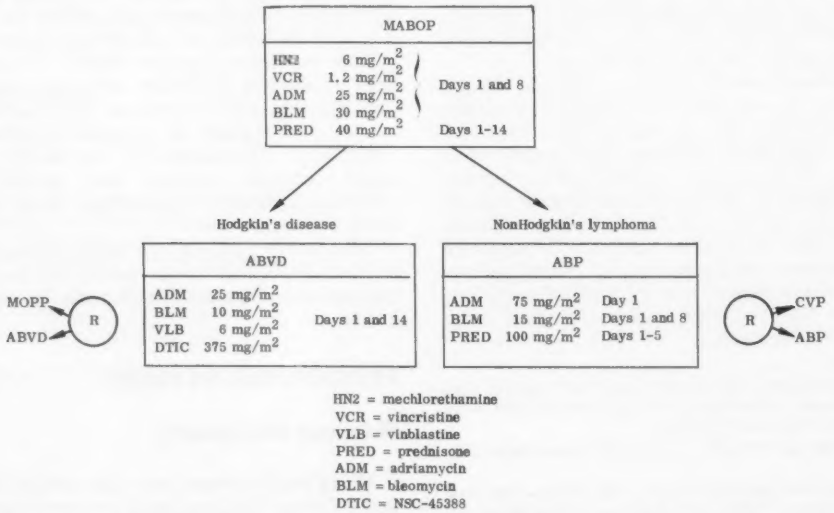

FIGURE 1.—Outline of chemotherapy combinations in the treatment of Hodgkin's disease and nonHodgkin's lymphoma.

nonHodgkin's lymphomas. ABVD and ABP were randomly tested against MOPP and cyclophosphamide, vincristine, and prednisone (CVP), respectively, to explore the comparability of therapeutic effects as well as cross resistance. Figure 2 shows the outline of study for ABVD and ABP. Stratification determinants included histopathologic subtypes, clinical and pathologic stages, and prior and no prior radiotherapy. It is important to note that in both studies, crossover was carried out in the presence of no response or progression after initial remission. Furthermore, in the ABVD versus MOPP study, low-dose (2000-3000 rads) high-energy radiotherapy was also received by all patients with stage IV disease to sites of major pretreatment involvement, which included lungs, liver, and bones. In both Hodgkin's disease and nonHodgkin's lymphomas, patients with stage IV disease who achieved complete remission after the first six cycles of chemotherapy were given six more cycles with a longer interval of time (2 months) between the two treatments. If after a total of 12 cycles patients were still considered to be in complete remission, no further chemotherapy was administered.

Dose Modification

As reported in previous publications (8,10,14,15), a dose-attenuation schedule was used when myelosuppression, severe stomatitis, or severe neurotoxicity occurred. In the majority of patients, the dose adjustments were carried out on the basis of blood cell counts obtained before starting each course. In fact, all patients were treated on an outpatient basis. Only in the first few patients of each series were the nadirs of the leukocyte and platelet counts systematically determined. This fact should be taken into consideration in the evaluation of the percent of patients with leukopenia and thrombocytopenia.

Assessment of Results

A patient was considered to be in a state of complete remission (CR) when all symptoms and signs of disease, as determined by roentgenographic and biochemical studies, had disappeared. In evaluating the therapeutic results of MABOP, patients with a 75% or greater (but not complete) reduction in the size of retroperitoneal adenopathies were considered to be in CR provided all other signs of disease had disappeared. In two recent trials (ABVD versus MOPP and ABP versus CVP), more strict criteria were employed to define CR (15). In fact, in patients with stage IV disease a second biopsy of accessible extralymphatic sites of known involvement such as the liver and bone marrow had to be interpreted as negative. Partial remission (PR) was defined as a greater than 50% reduction in the product of the longest perpendicular diameters of all evident disease. Patients with regressions less than 50% (objective improvement) or no change in measured tumors by the end of the sixth cycle were categorized as having failed to respond to induction therapy.

RESULTS

MABOP

Tables 2 and 3 show the therapeutic effects of MABOP after six cycles in patients with Hodgkin's disease and nonHodgkin's lymphomas. The results appear comparable in terms of total CR and CR + PR. As expected, in the limited number of patients who previously received chemotherapy, the percent of CRs was low. However, those who relapsed after prior local or extensive radiotherapy achieved a

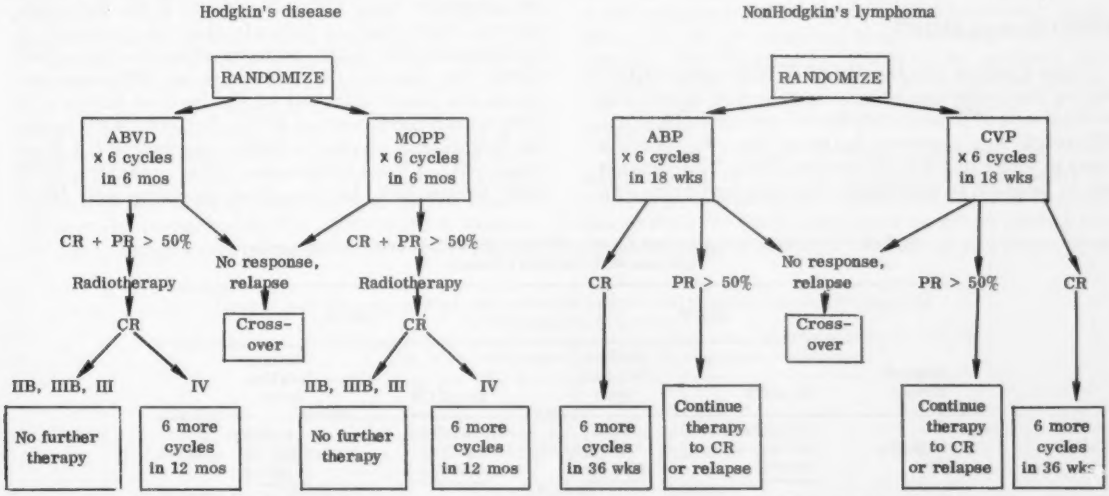

FIGURE 2.—Outline of controlled studies in Hodgkin's disease (stages IIB, IIIB, III_S, and IV) and in nonHodgkin's lymphoma (stage IV).

TABLE 2.—Complete remission after six cycles of MABOP in patients with Hodgkin's disease

Stage of disease	No. of evaluable patients	Total No. of patients responding/ total No. receiving— No prior therapy	Prior radiotherapy	Prior chemotherapy*	Total No. of CR	Total No. of CR + PR	Median duration of CR (mos)
IIB	11	6/8(75%)	2/3(66%)	—	8(73%)	9(82%)	21(5-44+)
IIIB, III_S (A + B)	30	16/19(84%)	6/7(86%)	3/4(75%)	25(83%)	28(93%)	21(1-43+)
IV (A + B)	20	2/8(25%)	3/6(50%)	1/6(17%)	6(30%)	11(55%)	13(12-32+)
Totals	61	(69%)	(69%)	(40%)	(64%)	(79%)	

*With or without radiotherapy.

TABLE 3.—Complete remission after six cycles of MABOP in patients with nonHodgkin's lymphoma

Stage of disease	No. of evaluable patients	Total No. of patients responding/ total No. receiving— No prior therapy	Prior radiotherapy	Prior chemotherapy*	Total No. of CR	Total No. of CR + PR	Median duration of CR (mos)
I_E	3	1/1	0/2	—	1(33%)	2(66%)	1
II_E, IIB	7	1/1	2/3	1/3	4(57%)	6(86%)	31(13-34)
IIIB, III_S (A + B)	22	10/14(71%)	7/8(87%)	—	17(77%)	18(82%)	12(2-41+)
IV (A + B)	24	7/11(64%)	3/8(37%)	1/5	11(46%)	15(62%)	8(1-41+)
Totals	56	(70%)	(57%)	(25%)	(59%)	(73%)	

*With or without radiotherapy.

percent of CR identical or similar to that achieved by untreated patients. In Hodgkin's disease, there was no difference in the median duration of CR between patients with stage II and III disease who had also received radiotherapy after induction chemotherapy. The median duration of CR was 13 months for patients with stage IV disease. In patients with nonHodgkin's lymphomas, the median duration of response was longer in those with lymphocytic and nodular lymphomas than in those with histiocytic and diffuse types (14).

ABVD Versus MOPP

Table 4 shows the results of ABVD versus MOPP during the induction phase. As reported in previous evaluations of a small number of patients (10,15), no difference was observed between the two combinations in terms of CR (74% versus 77%). The comparison in relation to histologic subtypes and prior irradiation also failed to reveal a significant difference between the two treatment groups and indicated that the percent of CR was not influenced by previous radiotherapy. The preliminary analysis of the median duration of CR provides a further indication that ABVD appears to be as effective as MOPP.

ABP Versus CVP

A comparison of responses to ABP and CVP is reported in table 5. Both treatments yielded a similar percent of total CR (44% versus 41%). However, present data seem to indicate that in lymphocytic lymphomas CVP (55%) is more effective than ABP (36%). On the contrary, there is no difference between the two treatments at the level of histiocytic lymphomas (38% versus 43%). Patients with nodular lymphomas showed a higher percent of CR than those with diffuse lymphomas after receiving CVP (60% versus 40%) but not after receiving ABP (40%

TABLE 4.—Comparative response between MOPP and ABVD as primary therapy for patients with Hodgkin's disease

Stage of disease	MOPP No. of CR	MOPP Median duration (mos)	ABVD No. of CR	ABVD Median duration (mos)
IIB	3(100%)	4(2-7)	3(100%)	2.5(1-7)
IIIB, III_S	16(75%)	7(3-15)	18(72%)	7(2-12)
IV	16(69%)	10(4-15)	14(79%)	8(2-16)
Totals	35(74%)	7+(2-15)	35(77%)	7+(1-16)

TABLE 5.—Comparative response between CVP and ABP as therapy for patients with stage IV nonHodgkin's lymphoma

	CVP		ABP	
Histology	No. of CR	Median duration (mos)	No. of CR	Median duration (mos)
Histiocytic	13(38%)	6(3-10)	14(43%)	2.5(1-8)
Lymphocytic	11(55%)	5(1-14)	11(36%)	5(3-10)
Mixed type	1(0)	—	2(1%)	2
Totals	25(44%)		27(41%)	

versus 41%). At the time of the present analysis, the median duration of response in patients with the histiocytic type appeared to be longer after CVP than after ABP. No difference was observed in patients with lymphocytic lymphomas.

Crossover

The results of crossover for patients with progressive disease are recorded in table 6. At the time of this writing, most patients were still receiving the secondary treatment; therefore, no conclusive data can as yet be provided about the quality of the response. Available data indicate an absence of cross resistance between treatments, although secondary therapy with ABP yielded only objective improvement in two of eight patients.

Toxic Effects

A comparative evaluation of the principal toxic manifestations observed with each of the combinations employed is presented in table 7. As mentioned before, the peripheral blood cell count was determined only on the day the drug was administered. The highest incidence of leukopenia was observed with MABOP, and the highest incidence of thrombocytopenia was observed with ABP. In general, thrombocytopenia occurred in a much lower percent of patients compared to leukopenia. The number of patients who suffered a loss of hair, though this effect was reversible, was high in all three combinations that included adriamycin as well as in the CVP regimen. Paresthesias were encountered more often in patients receiving the MOPP regimen (74%). Stomatitis was always moderate and did not require a dose-reduction schedule for adriamycin or bleomycin. Skin changes secondary to bleomycin (stria and cutaneous sclerosis of palms and fingers) occurred, especially after the MABOP regimen. The incidence of these skin changes is probably due to the higher single dose of bleomycin (30 mg/m^2) administered in this regimen compared to that administered in the ABP (15 mg/m^2) and the ABVD (10 mg/m^2) regimens. Interstitial lung lesions were seen only in patients who received MABOP or ABP. These lesions were always detected at a very early stage through repeated chest roentgenograms, and never produced symptoms and signs of pulmonary insufficiency. The median toxic dose was 142 mg/m^2 (range, 60-240 mg/m^2). Amenorrhea occurred, particularly in patients receiving the four- and five-drug combinations. Definite cardiomyopathy was seen in two patients receiving ABP in whom the total dose of adriamycin exceeded 600 mg/m^2 (range, 630-700 mg/m^2). One patient died at home because of acute cardiac failure. In a third patient who received a total dose of 600 mg/m^2, the diagnosis of cardiac toxicity remains uncertain because not all of the clinical signs and symptoms were present.

Drug-related deaths were due to either severe agranulocytosis or thrombocytopenia or both. Irreversible myelosuppression was not observed after the administration of MABOP, MOPP, or ABVD. A second tumor (acute myeloblastic leukemia), probably induced by combined chemotherapy and radiotherapy, was the cause of death in two patients who received MABOP (16). Finally, as mentioned before, one patient in the ABP group died because of cardiomyopathy.

The total average percent of the optimal dose administered in different combinations is shown in table 8. As can be seen, a minimum of 81% of the projected dose for each drug could be given during the first six cycles of therapy. Bleomycin and prednisone

TABLE 6.—Complete plus partial remission after primary and secondary therapy for patients with malignant lymphoma

	No. of patients responding/total No. of patients receiving—					
Therapy	MOPP	versus	ABVD	CVP	versus	ABP
Primary	32/35(91%)		31/35(89%)	17/25(68%)		22/27(81%)
Secondary	4/5(80%)		4/6(66%)	3/7(43%)		2/8(25%)

TABLE 7.—Percent of toxicity related to the different chemotherapy combinations used

Side effects	No. of patients receiving—				
	MABOP (51 patients)*	MOPP (35 patients)	ABVD (35 patients)	CVP (25 patients)	ABP (27 patients)
Leukopenia (wbc <4000/mm³)	81	60	71	68	52
Thrombocytopenia (platelets <150,000/mm³)	21	25	11	24	30
Loss of hair	100	31	71	92	92
Stomatitis	21		3	4	25
Paresthesias	49	74	3	56	
Skin changes	72		46		48
Lung fibrosis	2		0		15 (median dose 142 mg/m²)
Cystitis				8	
Amenorrhea	39	20	46	4	0
Cardiomyopathy	0		0		7 (median dose 665 mg/m²)
Drug-related deaths	4	0	0	8	15
Infection	0			4	7
Hemorrhage	0			4	4
Both infection and hemorrhage	0			4	0
Second tumor	4	0	0	0	0

*Patients with Hodgkin's disease previously untreated with chemotherapy.

were not always administered at a full dose level because of definite or suspicious lung toxicity and because of gastric disturbances, respectively.

New Trials

On the basis of the above-described results, new studies were designed in September 1974. An outline for these studies is presented in figure 3.

During recent past experience with MOPP versus ABVD followed by radiotherapy (mantle-field irradiation, total or subtotal lymphoid irradiation), we have encountered a number of difficulties in carrying out the radiotherapy program within the optimal period of time. Myelosuppression and especially prolonged thrombocytopenia were common when irradiation was delivered to adenopathies below the diaphragm. A three-arm study was designed for use in patients with advanced Hodgkin's disease with nodal involvement. The control arm consists of radiotherapy followed by six cycles of MOPP as carried out at Stanford University (17). In the other two arms, three cycles of chemotherapy with either MOPP or ABVD are given before radiotherapy which is subsequently followed by three more cycles of either combination. This treatment schedule is somewhat similar to that reported by Prosnitz et al (18).

Patients with stage IV Hodgkin's disease were randomly allocated to 12 cycles of either MOPP or MOPP plus ABVD with the specific intent of explor-

TABLE 8.—Percent of optimal dose administered in different combinations

Drug	Average percent of total course received by patients on—				
	MABOP*	MOPP	ABVD	ABP	CVP
Mechlorethamine	87	89			
Cyclophosphamide					81
Procarbazine		89			
Vincristine	92	88			91
Vinblastine			87		
Adriamycin	88		87	82	
Bleomycin	97		96	85	
NSC-45388			96		
Prednisone	99	100		94	95

*Calculated only in patients with Hodgkin's disease previously untreated with chemotherapy.

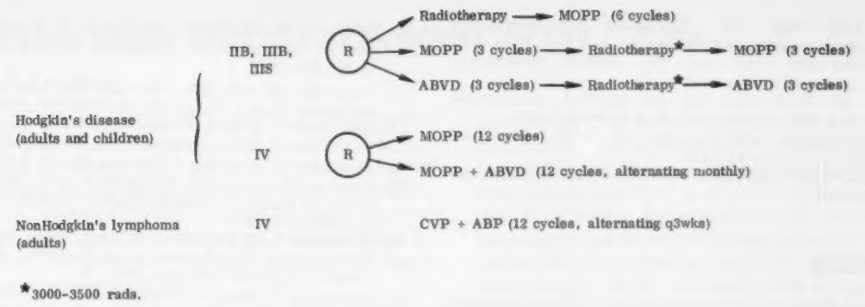
Radiotherapy → MOPP (6 cycles)
IIB, IIIB, IIIS
R
MOPP (3 cycles) → Radiotherapy* → MOPP (3 cycles)
ABVD (3 cycles) → Radiotherapy* → ABVD (3 cycles)
Hodgkin's disease (adults and children)
IV
R
MOPP (12 cycles)
MOPP + ABVD (12 cycles, alternating monthly)
NonHodgkin's lymphoma (adults)
IV
CVP + ABP (12 cycles, alternating q3wks)
*3000-3500 rads.

FIGURE 3.—New ongoing studies in advanced malignant lymphoma.

ing whether the monthly alternating schedule could improve the percent of long-term survivors free of disease.

The concept of alternating cycles with two noncross-resistant combinations is now also being applied in patients with nonHodgkin's lymphomas. Since the incidence of both CR and long-term disease-free survivors is low in this group of diseases, a control arm was not felt to be necessary.

COMMENTS

The results obtained so far with our trials can be summarized as follows.

A.—Adriamycin, as a single agent, appears to be effective in all types of malignant lymphomas and particularly in histiocytic lymphoma. Both the quality and the duration of response probably reflect prior chemotherapy. The results observed in our series, which are in agreement with those reported by other investigators (3,4,6,7), show an absence of cross resistance between adriamycin and conventional drugs useful in lymphomas.

B.—The results of three protocols of multiple-drug treatment which include adriamycin show that, in terms of remission induction, the remission rate obtained with six cycles of these new combinations appears to be comparable with the remission rate obtained with conventional treatment such as MOPP and CVP. In patients with nonHodgkin's lymphomas the percent of CR occurring after the ABP and CVP regimens probably could have been higher than that observed in our series if treatment had not been restricted to patients with stage IV disease. This statement is supported by the encouraging results (CR 57%-63%) of Schein et al (19), McKelvey et al (20), and Skarin et al (21) who in addition to treating patients with stage IV disease, used different combinations to treat patients with stage II and III disease.

C.—Although preliminary, available data show an absence of cross resistance between treatment groups. A more definite opinion about the quality of response after secondary treatments awaits further evaluation. In our opinion, however, there is sufficient evidence to explore the feasibility and the usefulness of alternating treatment cycles in patients with Hodgkin's disease and patients with non-Hodgkin's lymphomas in an attempt to improve the percent of CR and the number of disease-free survivors.

D.—Our results prove once more that adriamycin, when given in combination with conventional drugs, can be safely administered to most patients provided the total dose does not exceed 600 mg/m^2 (22). When, as in our experience, patients with good PRs require prolonged treatment, one should be aware of the potentially severe side effects of adriamycin. The same precaution applies to the use of bleomycin, although lung toxicity is, to a certain extent, less predictable. Therefore, in patients requiring prolonged treatment, it is advisable to continue therapy with another effective noncross-resistant combination.

In conclusion, MABOP, ABVD, and ABP appear to be effective combinations, especially in terms of remission induction. The results obtained so far encourage their use in combined treatment modalities as well as in combined sequential treatments with conventional multiple-drug regimens. The potentially severe, and to a certain extent, unpredictable side effects of both adriamycin and bleomycin, make long-term therapy difficult and even hazardous when these drugs are administered at full dose.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
ABP: adriamycin, bleomycin, and prednisone
ABVD: adriamycin, bleomycin, vinblastine, and NSC-45388
Bleomycin: NSC-125066
CVP: cyclophosphamide, vincristine, and prednisone
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
MABOP: mechlorethamine, adriamycin, bleomycin, vincristine, and prednisone
Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; HN2
MOPP: mechlorethamine, vincristine, prednisone, and procarbazine

NSC-45388: CAS reg. No. 4342-03-4; 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide
Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,20-dihydroxy-
Procarbazine: NSC-77213; CAS reg. No. 491-88-3; p-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride
Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); Oncovin

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Adriamycin (NSC-123127)—Thoughts for the Future[1]

Stephen K. Carter[2,3]

One of the most impressive aspects of adriamycin as an antitumor agent is its broad spectrum of activity (table 1). There are nine tumor categories in which adriamycin has established anticancer activity. These encompass a broad range of solid tumors that in the past have been relatively insensitive to chemotherapy, especially the soft tissue and bone sarcomas and bladder cancer.

There are seven additional tumor categories for which available data indicate some degree of adriamycin activity although a definitive statement cannot be made at this time. This group also includes some classically unresponsive tumor types such as gastric and prostatic carcinomas. Only three tumor categories can be said to be unresponsive to adriamycin. They include some of the tumors that are least sensitive to chemotherapy in general, eg, renal cancer and malignant melanoma. Despite the extensive clinical testing of adriamycin there are still five categories for which no adequate data exist. This will soon be rectified since various cooperative groups have nearly all of these tumors under phase II evaluation with adriamycin.

When an investigational drug is shown to be active in phase II evaluations, a study approach is established involving both the clinical trials area and the preclinical laboratories. In clinical studies the new drug is combined with other active drugs or integrated into established combinations in an attempt to increase the therapeutic activity against advanced disease. In addition, in keeping with the overall thrust of combined modality attacks against cancer (1), a new active drug is combined with surgery and/or radiotherapy and/or immunotherapy in an effort to increase the remission rate for primary therapy. At the same time, a range of approaches are taken in an attempt to improve the therapeutic index. These approaches include (*a*) schedule manipulation; (*b*) dosage manipulation; (*c*) pharmacologic investigations; (*d*) attempts to block toxicity; and (*e*) clinical experience. Finally, a vigorous search for analogs is made to find a new drug with greater efficacy and/or diminished toxicity.

Adriamycin has been used in a wide range of dose schedules (table 2) but efforts to improve the therapeutic index have not been highly successful. From the standpoint of efficacy, it is impossible with the available data to state that any one schedule is superior. There is a nearly total lack of controlled comparisons of schedules but the uncontrolled data appear to indicate rough comparability of efficacy for all the commonly used schedules. Dr. Yvon Kenis was among the first to report that a high single dose every 3 weeks gave diminished stomatitis, a fact also observed by other investigators. Currently, the recommended schedule is 60-75 mg/m^2 administered as a single rapid infusion and repeated every 21 days. This approach is supported by the pharmacokinetic studies in man reported by Drs. Benjamin and Bachur (2,3).

This paper will review the current status of studies with adriamycin in some of these areas in the hope of complementing the detailed presentations in this symposium.

CURRENT STATUS OF ADRIAMYCIN THERAPY

The current status of adriamycin combination regimens and combined modality approaches is outlined in table 3.

Breast Cancer

The initial studies with adriamycin revealed that it is one of the most active single agents for the treatment of metastatic adenocarcinoma of the breast. A 36% response rate in 121 evaluable patients was seen in the initial cumulative data review (4). This efficacy was particularly impressive since most of the treated patients had failed to respond to prior chemotherapeutic approaches, most of which involved intensive combinations. Several new studies have examined adriamycin as initial chemotherapy for advanced breast cancer in comparison to combination regimens. The results of these studies are particularly impressive (table 4).

Currently, most clinical groups are testing newly devised combinations including adriamycin in an attempt to increase the rate of remission induction and the duration of remission. At the NCI a newly devised three-drug combination (CAF) consists of: adriamycin (30 mg/m^2) intravenously (iv) on Days 1 and 8; cyclophosphamide (100 mg/m^2/day) orally × 14; and 5-fluorouracil (5-FU) (400 mg/m^2) orally on Days 1 and 8. Each cycle is repeated every 28 days. This is being compared to a regimen which consists of cyclophosphamide, methotrexate, and 5-FU (CMF) in which adriamycin is replaced by methotrexate at a dose of 40 mg/m^2 iv on Days 1 and 8 (8). Five of eight patients have responded to CAF, compared to four of ten patients who responded to CMF. This encouraging study, which suggests that adriamycin can be successfully integrated into a combination regimen, is continuing to accrue patients.

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[1]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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TABLE 1.—Tumor activity spectrum of adriamycin

Tumor responsiveness to adriamycin			
Established activity	Possible activity	Unresponsive	Data inadequate
Breast adenocarcinoma	Stomach adenocarcinoma	Large bowel adenocarcinoma	Pancreatic adenocarcinoma
Soft tissue and bone sarcomas	Ovarian adenocarcinoma	Malignant melanoma	Squamous cell carcinoma, esophagus
Bladder adenocarcinoma	Prostate adenocarcinoma	Renal cancer	Endometrial adenocarcinoma
Bronchogenic carcinoma	Squamous cell carcinoma, cervix		Brain tumors
Testicular carcinoma	Squamous cell carcinoma, head and neck		Chronic leukemias
Thyroid carcinoma	Hepatoma		
Pediatric solid tumors	Multiple myeloma		
Malignant lymphomas			
Acute leukemias			

TABLE 2.—Adriamycin dose schedules*

Institute	Investigator	Schedule
NCI, Milan	Dr. Gianni Bonadonna	a. 0.4, 0.6, or 0.8 mg/kg/day × 4; rest 3 days; then same doses weekly
		b. Same doses as in a every other day × 4-6 doses; then same doses weekly
		c. Above × 3 days; no treatment for 4 days; then treatment × 4 days
		d. × 2 days; no treatment for 7 days; repeat
		e. 25 mg/m²/day × 3, q3wks
Roswell Park Memorial Institute		a. 17.5-35 mg/m²/day × 2-3; no treatment for 3-4 days; repeat
		b. 20 mg/m²/day × 4
		c. 40 mg/m²/wk
M.D. Anderson Hospital		a. 20-30 mg/m²/day × 3, q3wks
		b. 20-35 mg/m²/wk × 9
		c. 60-105 mg/m², single dose q3wks
Sloan-Kettering Institute	Dr. Irwin Krakoff	0.5 mg/kg/day × 3; rest 4 days; same dose × 3 days, etc, until toxicity or response occurs
	Dr. Charlotte Tan	a. 0.5 mg/kg/day × 4-6
		b. 0.25-0.5 mg/kg, q6h × 4
		c. 1 mg/kg/day × 2-3
		d. 2 mg/kg, single dose
Institut Jules Bordet	Dr. Yvon Kenis	75 mg/m², single dose q3wks

*All administered iv.

TABLE 3.—Current status of adriamycin studies

Tumor	Studies in progress		
	Single-agent activity	Combination regimen superior to single agent	Combined modality role
Breast cancer	Yes	Yes*	Under test
Sarcomas			
Excluding osteogenic	Yes	Yes†	Under test
Osteogenic	Yes	Under test	Positive
Lung cancer	Yes	No	No
Malignant lymphomas	Yes	Yes (various drugs)	Under test
Bladder cancer	Yes	No	No
Testicular cancer	Yes	No	No
Thyroid cancer	Yes	No	No
Pediatric tumors	Yes	Under test	Under test
Acute leukemias	Yes	Yes	No

*Cyclophosphamide plus adriamycin; cyclophosphamide, 5-FU plus adriamycin.
†NSC-45388 plus adriamycin; NSC-45388, cyclophosphamide, vincristine plus adriamycin.

TABLE 4.—Adriamycin alone vs combination chemotherapy in the treatment of advanced breast cancer

Investigators (ref No.)	Adriamycin alone		Combination regimen	
	Dose schedule	Response	Dose schedule	Response
Rosner et al (5)	75 mg/m² q3wks	8/23*	Every 28 days: a. 5-FU (8 mg/kg/day × 5) b. Cyclophosphamide (4 mg/kg/day × 5) c. Prednisone (10 mg twice daily × 14)	13/25*
Hoogstraten et al (6)	60 mg/m² q3wks	55%	Cooper regimen: a. Continuous b. Intermittent	65% 59%
Ahmann et al (7)	60 mg/m² q3wks	9/20*	FCP: a. 5-FU (8 mg/kg/day × 5) b. Cyclophosphamide (4 mg/kg/day × 5) c. Prednisone (30 mg/day) FCP+ vincristine (1.4 mg/m² Days 1 and 5)	7/16*

*No. of patients responding/No. of patients treated.

Another regimen utilizing the same three drugs (FAC) has been developed at the M.D. Anderson Hospital (9). The drugs are administered on a 21-day course as follows: adriamycin (50 mg/m²) iv on Day 1; cyclophosphamide (500 mg/m²) iv on Day 1; and 5-FU (500 mg/m²) iv on Days 1 and 8. Twenty-five patients have been treated on this regimen with three complete remissions (CR) and 15 partial remissions (PR) being observed for a 72% response rate. Of 13 patients completing three courses, three patients had CRs and eight patients had PRs for a response rate of 84%. This pilot study, which had significant but acceptable toxic effects, reveals major efficacy in metastatic breast cancer and will be further evaluated in a cooperative group study.

Salmon and Jones (10) have developed a two-drug combination of adriamycin (40 mg/m² on Day 1) and cyclophosphamide (200 mg/m² orally on Days 3-6) with courses repeated every 21 days. They have treated 26 consecutive patients with advanced breast cancer and treatment was well tolerated with no serious myelosuppression. Of 23 evaluable patients, 19 received an adequate trial (two courses) and 16 of these showed excellent responses with greater than 50% tumor regression. Thus, the ratio of responders to evaluable trials was 16:23 (70%) and the ratio of responders to adequate trials was 16:19 (84%). Only one responder has relapsed to date with a remission duration of 3 months.

Thus, three combinations including adriamycin plus cyclophosphamide either alone or in further combination with 5-FU have shown high activity in breast cancer and further studies are being vigorously pursued. Currently, studies combining adriamycin with surgery in patients with poor risk for cure are being initiated.

Sarcomas

The overall response in sarcomas for adriamycin as a single agent has been reported as 26% (46 of 176 patients) (4), with a range of 10%-40% in individual studies. The variability of response rate as a function of cell type is demonstrated by the findings for bone sarcomas, including a 48% response rate (14 of 29 patients) in Ewing's sarcoma and a 35% response rate (11 of 35 patients) in osteogenic sarcoma, and for soft tissue sarcomas where response rates range from 30% to 36% (11-20). The combination approaches of Gottlieb et al (21) have been presented at this symposium (22).

Currently, nine different combinations utilizing adriamycin have been evaluated or are in progress in the metastatic sarcomas. They include:

Adriamycin + NSC-45388 (DTIC)
Adriamycin + methotrexate
Adriamycin +vincristine
Adriamycin +NSC-45388 +vincristine
Adriamycin +NSC-45388 + NSC-122819 (VM-26)
Adriamycin + vincristine +cyclophosphamide
Adriamycin +NSC-45388 + vincristine + cyclophosphamide
Adriamycin + actinomycin D + vincristine + cyclophosphamide
Adriamycin + actinomycin D +cyclophosphamide +vincristine (two-drug sequential)

Of particular interest are the studies of adriamycin used as a surgical adjuvant in osteogenic sarcoma in the hope of increasing cure rates in this dread tumor. Death from osteogenic sarcoma results from metastases in 80%-95% of patients, with roentgenographic evidence of pulmonary metastases occurring in a median of 9 months from initial diagnosis (23). The studies reported in this symposium, as well as earlier results (24, 25), indicate a positive role for adriamycin in this clinical situation.

At Childrens Hospital at Stanford University, Wilbur et al (26) have devised a treatment consisting of the following drugs given 3 weeks apart and cycled every 9 weeks: methotrexate (50-300 mg/kg) by infusion followed by citrovorum factor rescue; adriamycin (60 mg/m^2) iv plus vincristine (2 mg/m^2) iv; and cyclophosphamide (15 mg/kg/day × 7) iv or orally plus vincristine (2 mg/m^2) on Days 1 and 7. Four of five patients who received this regimen after amputation are free of disease at 12+, 12+, 10+, and 7+ months.

At this time, the potential of adriamycin alone or in combination with other drugs to increase survival when used as an adjuvant to surgery in osteogenic sarcoma appears quite high.

The effectiveness of adriamycin (26% of 229 patients) (4) relative to other single agents can be evaluated on the basis of objective response but the adriamycin data are insufficient to judge survival as a parameter of response. Within the limitations of historical comparisons, and including the less than 50% regressions, adriamycin ranks among the most effective drugs reported from the data base of over 5000 patients used by Selawry and Hansen (27, 28); ie, mechlorethamine (36%), CCNU (27%), methotrexate (25%), cyclophosphamide (23%), and hexamethylmelamine (20%).

Bronchogenic Carcinoma

Adriamycin is being evaluated in a variety of combinations in an attempt to increase the response rates in bronchogenic carcinoma. The Veterans' Administration Lung Cancer Study Group is evaluating adriamycin plus cyclophosphamide, while the Central Oncology Group is conducting a study of adriamycin (15 mg/m^2/week) plus CCNU (70 mg/m^2 given orally every 6 weeks) plus hexamethylmelamine (100 mg/m^2 given orally on Days 3-5). At the M.D. Anderson Hospital an intensive five-drug combination (BACON) has been developed by Livingston et al (29) with a weekly regimen as follows:

Day 1 and then every 4 weeks:
Adriamycin (40 mg/m^2 iv)
CCNU (65 mg/m^2 orally)
Mechlorethamine (8 mg/m^2 iv)

Weekly to a total dose of 180 mg of bleomycin:
Vincristine (0.75-1.0 mg iv on Day 2)
Bleomycin (30 mg iv 6 hours after vincristine).

Lowenbraun (30) at the University of Louisville, has treated 28 patients with metastatic lung cancer with a monthly regimen as follows:

Day 1:
Cyclophosphamide (600 mg/m^2 iv)
Adriamycin (60 mg/m^2 iv)
NSC-45388 (250 mg/m^2 iv)
Days 8 and 15:
Vincristine (2 mg iv)
Days 22 and 24:
Hydroxyurea (2 g/m^2 orally)
Methotrexate (20 mg/m^2 orally).

Among 15 patients with undifferentiated lesions, 12 of 13 evaluable patients responded. Among 11 patients (eight evaluable) with squamous cell carcinoma, there were only one good and two questionable responders; two patients with adenocarcinoma were questionable responders.

Malignant Lymphoma

Adriamycin has shown significant activity against all of the malignant lymphomas with an overall response rate of 41% (61 of 147 patients) (4). The response rate was 36% (23 of 64 patients) in Hodgkin's disease, 56% (19 of 34 patients) in reticulum cell sarcoma, and 34% (12 of 35 patients) in lymphosarcoma (11, 14, 16, 17).

Adriamycin could be considered inferior by retrospective comparison with most other active agents (31-33). However, most of the adriamycin-treated patients had advanced disease and probably had failed standard therapy including combination chemotherapy. In this type of patient population the response rate observed could be significant because it suggests a very low level of cross-resistance between adriamycin and other agents. Thus, adriamycin has been considered for use in patients refractory to other drugs and for inclusion in new combined therapy approaches.

Besides the Southwest Oncology Group (SWOG) studies reported earlier by McKelvey et al (34), Skarin et al (35) at the Sidney Farber Cancer Center in Boston have developed a regimen (BACOP) for non-Hodgkin's lymphoma which consists of:

Bleomycin: 4 mg/m^2 iv twice weekly for 5 weeks
Adriamycin (45 mg/m^2) and cyclophosphamide (600 mg/m^2): iv Days 1, 22, 43
Vincristine: 1.2 mg/m^2/wk × 7
Prednisone: 40 mg/m^2/day × 28.

Of 19 evaluable patients treated, CR was achieved in four of six patients (67%) with nodular lymphoma and in eight of 13 patients (62%) with diffuse lymphoma. Among 12 patients with CR, two have relapsed at 7 and 8 months, while ten patients have been disease-free for 2-11 months (median 4+ months).

Acute Leukemia

Adriamycin has been active against both types of acute leukemia, with a 24% CR rate (47 of 195 patients) in refractory patients (4). By cell type, 36 of 148 patients (24%) with acute lymphocytic leukemia (ALL) had CR and the overall response rate was 39% (58 of 148 patients). In patients with acute myelocytic leukemia (AML), the CR rate was 23%.

At the M.D. Anderson Hospital, adriamycin has been integrated into a combination regimen for adult acute leukemia (36). Thirty-three patients have received adriamycin (40 mg/m^2) given iv on Day 1 followed on Day 5 by cytosine arabinoside (100 mg/m^2 × 5 days) as a continuous iv infusion, with vincristine (2 mg/day) given iv on Day 1 and prednisone (100 mg/day) given on Days 1-5. All patients were evaluated immunologically before therapy and bacillus Calmette-Guérin was administered on Days 12 and 17 of each course and by the scarification technique prior to therapy, where possible. The second course was started on Day 19 with the appropriate dose adjustment depending on bone marrow cellularity and blast cell count. Twenty-six patients had AML and there were seven patients with acute undifferentiated leukemia or ALL. Twenty-three patients (70%) achieved CR and 27 patients had an adequate trial of therapy with CR in 85% of these patients. Myelosuppression was the major toxic effect and the second course was delayed when the bone marrow cellularity was less than 10%. Thirty-two of 33 patients (97%) received prophylactic platelet transfusions and 27 patients received granulocyte transfusions for infectious episodes during remission induction. Where supportive care is available, this regimen is a highly effective approach to remission induction in adult acute leukemia.

Other Tumors

Adriamycin has shown activity against a range of genitourinary tract tumors (4). The overall response rate in bladder cancer is 33% (17 of 52 patients) and, on the basis of published reports, 5-FU (35% response, 26 of 74 patients) is the only other agent that has been adequately studied (37). The data for testicular tumors are predominantly in the nonseminomatous type where the overall response rate is 18% (seven of 39 patients). The SWOG has reported (16) two responses to adriamycin in nine patients with adenocarcinoma of the prostate, and other studies are currently under way to develop additional data.

In multiple myeloma, the SWOG has obtained two responses with adriamycin in 21 patients with advanced disease who had failed other therapy (16). This same group is currently studying adriamycin in combination with melphalan and prednisone with a regimen consisting of: adriamycin (25 mg/m^2), melphalan (6 mg/m^2/day orally × 4), and prednisone (60 mg/m^2/day orally × 4). This regimen is repeated every 4 weeks.

Adriamycin has been studied more extensively than any other antineoplastic agent in thyroid cancer. Thus far, the response rate is 45% (ten of 22 patients) but analysis by cell type is not available. Gottlieb's review of chemotherapy in thyroid tumors points out the limited accounts reported in the literature (38). Adriamycin could be considered the drug of choice for progressive metastatic thyroid cancer on the basis of its known activity and the lack of positive data for other agents. Adriamycin may become the reference drug for trials of more conventional chemotherapeutic agents.

ANALOGS

As soon as the dramatic activity of adriamycin was elucidated, a vigorous search for analogs was begun by the Drug Research and Development (DR&D) program of the Division of Cancer Treatment (DCT), NCI. This has involved a wide range of compounds developed in western Europe and the USSR. Twelve of these compounds, including six that have undergone some degree of clinical trial abroad, have been evaluated by the Laboratory of Experimental Chemotherapy, DR&D, DCT (table 5).

Among the analogs with the most clinical data available is rubidazone, a benzoylhydrazone derivative of daunorubicin. In experimental tumor systems rubidazone is more active than daunorubicin but is

not more effective than adriamycin.[4] Rubidazone is less toxic than adriamycin and similar to daunorubicin in cardiac toxicity studies in the hamster. In other in vitro and in vivo animal tests, rubidazone is less toxic than either daunorubicin or adriamycin (39). Studies in the rabbit cardiac toxicity model (40) indicate that rubidazone does cause cardiac toxic effects but at a higher total dose (mg/m^2) than adriamycin.[5] This could be deceptive in predicting toxicity for man since much higher doses are used in a single course of rubidazone treatment when compared with adriamycin (41-43).

The first clinical studies with rubidazone were performed at the Hôpital Saint-Louis in Paris. These studies were restricted to acute leukemia and reported results showed 11 of 24 patients with CR in ALL and 17 of 32 patients with CR in AML, including 12 responders among 18 patients without prior chemotherapy. The dose schedule has been 3.5 mg/kg (110-185 mg/m^2) daily for 4-7 days to obtain a total dose of 20-25 mg/kg (750 mg/m^2). As many as three doses of chemotherapy could be repeated at the point of bone marrow aplasia if blast cells persisted.

Nothing can be said concerning dose-related cardiac toxic effects since maintenance therapy with rubidazone was not attempted. However, electrocardiographic changes have been observed in only one trial in solid tumors in France (44). Acute toxic effects of rubidazone are similar to those of adriamycin, with alopecia occurring in all patients (43), and nausea, vomiting, diarrhea, and rash occurring in a few patients. Marrow toxic effects are the limiting toxic effects although Jacquillat reports that rubidazone produces a smoother induction with fewer deaths from toxic effects in comparison to his experience with daunorubicin. Clinical trials with this compound will soon be initiated in the US.

Both duborimycine and daunomycin-13-formylhydrazone have received some clinical evaluation in France but are clinically less impressive than rubidazone.[6]

Carminomycin is an analog of daunorubicin originally isolated from the mycelium of *Actinomadura carminata* by Brazhnikova et al (45) at the Institute of New Antibiotics, Academy of Medical Sciences, Moscow, USSR. The structure (fig 1) has been described by Brazhnikova et al (46). Experimental studies in the US show that carminomycin has significant antitumor activity but lacks superiority to adriamycin in all systems studied.[7] Clinical trials now in progress in the USSR show an acute toxicity spectrum similar to that of other drugs in this class.[8]

The complexing of adriamycin or daunorubicin with DNA is based on the hypothesis of DeDuve and Trouet that DNA confers lysosomotropic properties on the drugs and enables them to achieve selectively higher incorporation into the neoplastic cells (47, 48). Experimental tumor studies in Belgium and at the NCI have indicated potentially fewer toxic effects and increased efficacy for the DNA complexes. The experimental picture has been complicated by an NCI formulation of adriamycin-DNA showing less activity than the original formulations of Trouet and his associates, and the NCI is now attempting to prepare a new formulation. Clinical studies with both complexes have been undertaken in Europe but their preliminary nature precludes any judgment at this time. Clearly, additional work should be vigorously pursued on this highly interesting approach.

The most critical questions in analog searching after a drug is shown to be active are: (*a*) what is the basis for choosing a new analog for clinical evaluation, and (*b*) how should its clinical trial be approached? Three basic approaches are being considered for adriamycin analogs. Experimental tumor data are the most commonly used tools in choosing a new analog in most drug development programs. The same experimental tumor systems used to predict activity of the parent compound can be employed to find superior activity for an analog. This is most easily accomplished when the parent compound is only weakly or moderately active, as is the case with adriamycin in the L1210 leukemia system. It becomes more difficult when the parent compound is already curative to a high degree in a system, such as adriamycin in B16 melanoma and P388 leukemia. A second experimental design would be to screen for activity in systems completely unresponsive to adriamycin. This approach is taken in many analog drug development programs which use panels of tumors to search for differential tumor spectra (49).

A toxicologic model could also be incorporated in the decision-making process for choosing an adriamycin analog for clinical trial. Since dose-related cardiac toxic effects are the major limitation to the protracted use of adriamycin, a drug with diminished potential for cardiac toxicity, even with less efficacy, would be highly desirable. Potential models in the rabbit, monkey, hamster, and rat have been postulated to have some degree of predictive value and all will be looked at in further detail.

The third major approach in choosing an analog is to examine the data base of clinical experience in other countries. As stated earlier, six analogs of adriamycin and daunorubicin have been clinically evaluated abroad and the information on activity and toxic effects will obviously play a major role in deciding which ones might undergo widespread trial in the US.

After an analog has been selected for clinical trial, the strategy of testing becomes of paramount importance and must be designed from the standpoint of both efficacy and toxicity (fig 2). The efficacy of the analog could be compared to adriamycin in tumors either highly or moderately responsive to

[4]Goldin A. Personal communication.
[5]Young D. Personal communication.
[6]Jacquillat C. Personal communication.
[7]Goldin A. Personal communication.
[8]Perevodchikova N. Personal communication.

adriamycin or totally unresponsive. The major problem with highly responsive tumors is that drug combinations involving adriamycin have the highest priority within the treatment strategy for these tumors. It would be unrealistic to expect a valid test of an analog in patients previously treated with adriamycin in a combination setting. It might be equally unrealistic to pursue a single-agent trial with an unproven analog where superior combinations are known to exist. The same problems arise to a lesser degree in moderately responsive tumors and it is not unrealistic at all to contemplate a single-agent trial with an analog in previously untreated patients with bronchogenic carcinoma. The tumors that are unresponsive to adriamycin are relatively easy to use for analog testing, but used alone they might not constitute a fair trial of the predictive ability in the decision-making process.

When evaluation of the cardiac toxicity of a new analog is brought into focus the problems are manifold. One could maintain patients who are responsive to an analog as long as relapse is prevented and observe for congestive heart failure. This is what occurred with adriamycin in the face of the knowledge of the toxic potential of daunorubicin. Although "ignorance is bliss" to some extent, a continued approach in this manner might not be defensible. An attempt could be made to use electrocardiographic or other noninvasive measurements, but their past value in predicting for the chronic toxicity has been questionable.

TABLE 5.—Daunorubicin and adriamycin analogs under evaluation in comparison to parent compounds

NSC No.	Compound	Source
18334	Cinerubin A	Ciba
18335	Cinerubin B	Ciba
100289	Rutilantin	Dr. Ulrich Weiss
102813	β-Rhodomycin	E. R. Squibb
109351	Daunomycinone	Stanford Research Institute
149584	Adriamycin-14-octanoate	Farmitalia (Italy)
164011	Rubidazone*	Rhone-Poulenc (France)
169533	Daunomycin-DNA complex*	Belgium
169534	Adriamycin-DNA complex*	Belgium
180024	Carminomycin*	USSR
180510	Duborimycine*	Rhone-Poulenc (France)
180511	Daunomycin-13-formylhydrazone*	Rhone-Poulenc (France)

*Compounds that have undergone some degree of clinical trial abroad.

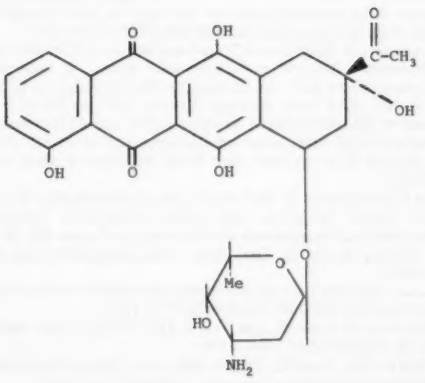

FIGURE 1.—Carminomycin structure.

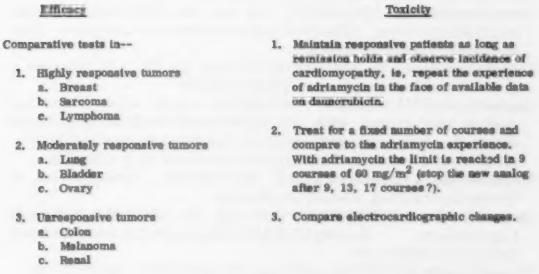
Efficacy
Comparative tests in—
1. Highly responsive tumors
a. Breast
b. Sarcoma
c. Lymphoma
2. Moderately responsive tumors
a. Lung
b. Bladder
c. Ovary
3. Unresponsive tumors
a. Colon
b. Melanoma
c. Renal
Toxicity
1. Maintain responsive patients as long as remission holds and observe incidence of cardiomyopathy, ie, repeat the experience of adriamycin in the face of available data on daunorubicin.
2. Treat for a fixed number of courses and compare to the adriamycin experience. With adriamycin the limit is reached in 9 courses of 60 mg/m2 (stop the new analog after 9, 13, 17 courses?).
3. Compare electrocardiographic changes.

FIGURE 2.—Factors to be considered in evaluating new analogs for chemotherapeutic effectiveness.

CONCLUSION

Adriamycin is a drug with a wide spectrum of antitumor activity. The logical development of clinical trials in responsive tumors has moved from single-agent studies to combination studies which involve other drugs active against the particular tumor. Of particular interest are the combinations with cyclophosphamide that have already shown promising results in breast cancer, the sarcomas, and non-Hodgkin's lymphomas, and are currently under study in lung cancer. Combined modality studies involving surgery and/or radiotherapy are actively under way in osteogenic sarcoma and are the place where this exciting new drug will probably find its ultimate role in cancer treatment.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
Adriamycin-DNA complex: NSC-169534; nucleic acid deoxyribo-, sodium salt, compd. with (8*S-cis*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6, 8, 11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride
Adriamycin-14-octanoate: NSC-149584; adriamycin, 14-octanoate, hydrochloride
BACON: bleomycin, adriamycin, CCNU, vincristine (Oncovin), and mechlorethamine (nitrogen mustard)
BACOP: bleomycin, adriamycin, cyclophosphamide, vincristine (Oncovin), and prednisone
Bleomycin: NSC-125066
CAF: cyclophosphamide, adriamycin, and 5-fluorouracil
Carminomycin: NSC-180024; 5,12-naphthacenedione, 8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoxyl)oxy]-7,8,9,10-tetrahydro-1,6,8,11-tetrahydroxy-, (8*S-cis*)-
CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
Cinerubin A: NSC-18334
Cinerubin B: NSC-18335
Citrovorum factor: NSC-3590; CAS reg. No. 6035-86-5; glutamic acid, *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-, calcium salt
CMF: cyclophosphamide, methotrexate, and 5-fluorouracil
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan
Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride
Daunomycin-DNA complex: NSC-169533; nucleic acid, deoxyribo-, sodium salt, compd. with (8*S-cis*)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride
Daunomycin-13-formylhydrazone: NSC-180511; daunomycinone, 8-(formylhydrazone), monohydrochloride
Daunomycinone: NSC-109351; CAS reg. No. 27576-80-3; 5,12-naphthacenedione, 8-acetyl-7,8,9,10-tetrahydro-6,8,10,11-tetrahydroxy-1(or 4)methoxy-
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6; daunomycin
5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide: NSC-45388; CAS reg. No. 4342-03-4; DTIC
Duborimycine: NSC-180510; CAS reg. No. 26164-47-6; 5,12-naphthacenedione, 10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(1-hydroxyethyl)-1-methoxy-, (8*S-cis*)-, hydrochloride
FAC: 5-fluorouracil, adriamycin, and cyclophosphamide
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU
Hexamethylmelamine: NSC-13875; CAS reg. No. 645-05-6
Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1
Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard
Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
NSC-122819: demethylepipodophyllotoxin 9-(4,6-0-2-thenylidene-β-D-glucopyranoside); VM-26
Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,-11,20-trione, 17,21-dihydroxy-
β-Rhodomycin: NSC-102813
Rubidazone: NSC-164011; CAS reg. No. 36508-71-1; daunorubicin, benzoylhydrazone, monohydrochloride
Rutilantin: NSC-100289
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); Oncovin

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Tabular Analysis of the Clinical Chemotherapy of Solid Tumors

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The Division of Cancer Treatment (DCT) of the National Cancer Institute (NCI) is responsible for drug development and for monitoring clinical trials of new anticancer agents. A new function of the Division is the evaluation of combined modality approaches to cancer therapy with major emphasis on the solid tumors responsible for most cancer deaths in the United States. A recent review by Carter and Soper (1) outlined the strategy for integrating chemotherapy into combined modality approaches. The main thrust of this approach will be with chemotherapy plus surgery and/or radiotherapy and/or immunotherapy. Chemotherapy will involve the use of either single drugs or combinations of drugs. An evaluation of the potential of chemotherapeutic agents for use in successful combined modality therapy for any solid tumor requires consideration of two factors:

a. The number of drugs with different mechanisms of action that are active in the tumor, and
b. The degree of activity exhibited by the active agents, either alone or in combination.

In order to develop a data base for establishing priorities in advanced disease studies, Carter and Soper (1) outlined the available data pertinent to these considerations for 29 "standard" antitumor drugs in 16 major solid tumors.

The staff of the Cancer Therapy Evaluation Program (CTEP), DCT, NCI, recently made an intensive review of the analysis of Carter and Soper (1), which led to an updating and expansion of the data. In addition, it provided some cumulative numerical data to support the qualitative evaluations of each drug in each tumor type with regard to the adequacy of clinical data and the level of activity.

This analysis of the anticancer drugs in each of the major solid tumors is designed to identify those which are active, and therefore to provide the tools for combination chemotherapy and combined modality therapy. It also identifies the drugs and tumor types for which more data are needed. The analysis will also, as well as possible, provide some answers to the many inquires made to the CTEP regarding efficacy data for a specific anticancer drug or for many drugs in a particular tumor type.

METHODS OF REVIEW AND ANALYSIS

Table 1.1 indicates the sources of the 30 anticancer drugs used in the analysis. Drugs available from the NCI are supplied only as investigational drugs under NCI-sponsored Investigational New Drug (IND) applications. Three of these drugs have a New Drug Application (NDA) pending with the Food and Drug Administration, and if approved, will be commercially marketed in the United States. These are BCNU, CCNU, and L-asparaginase. Some of the drugs that are already commercially marketed have undergone clinical trials for non-approved indications, dosages, or routes of administration under an NCI-sponsored IND application or under one sponsored by an individual investigator.

Table 1.2 defines the activity symbols used in the tabular analysis of the drugs and diseases. The designation "NE" denotes drugs that have not been evaluated or situations where a drug has been inadequately evaluated and no decision can be made regarding its activity. The symbol "-" indicates that adequate evaluation has taken place and the drug is inactive. Drugs which have been evaluated to an extent showing evidence of activity, although it is not fully established, are indicated by "+," and drugs showing definite clinical activity are indicated by "++."

Table 2 is a broad-outline cross-reference chart showing the status of clinical evaluation for 30 drugs against 17 solid tumors based on published and unpublished results on file with the CTEP. Each of the 30 drugs has shown definite evidence of activity against at least one tumor type from among the hematologic malignancies or solid tumors. Investigational drugs, which have not been adequately evaluated or have shown no evidence of activity in any tumor, and hormonal agents are excluded from this review.

The question of whether evaluation of a particular drug in a specific tumor has been sufficient to allow a decision on its clinical activity is obviously an arbitrary one. Nevertheless, the drug-tumor interactions in table 2 have been characterized by the CTEP staff through analysis of what is felt to be the maximum data available to any single group experienced in evaluating anticancer agents.

Tables 3.1-3.21 group the data by disease, with the most to least active drugs listed; tables 4.1-4.30 regroup the data by drug, listing the tumors from the most to least responsive types. These tables contain the numerical data used in making the clinical evaluations in table 2. The figures provide the number of responders divided by the number of evaluable patients, and this fraction multiplied by 100 yields the percent response rate. A response rate was arbitrarily determined only when the number of evaluable patients was 15 or more.

The best available definition of an evaluable patient is one who has received the drug by an appro-

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TABLE 1.1.—Anticancer drugs evaluated

Classification	Drug	Abbreviation	Source
Alkylating agents	Cyclophosphamide	CYC	Mead Johnson Laboratories
	Mechlorethamine	HN2	Merck Sharp & Dohme
	Chlorambucil	CHL	Burroughs Wellcome Co.
	Melphalan	MPL	Burroughs Wellcome Co.
	Busulfan	BUS	Burroughs Wellcome Co.
Antimetabolites	5-Fluorouracil	5-FU	Roche Laboratories
	Methotrexate	MTX	Lederle Laboratories
	6-Mercaptopurine	6-MP	Burroughs Wellcome Co.
	6-Thioguanine	6-TG	Burroughs Wellcome Co.
	Cytosine arabinoside	Ara-C	Upjohn Co.
Mitotic inhibitors	Vincristine	VCR	Eli Lilly & Co.
	Vinblastine	VBL	Eli Lilly & Co.
Antitumor antibiotics	Actinomycin D	ACT	Merck Sharp & Dohme
	Mithramycin	MTH	Pfizer Laboratories
	Daunorubicin	DNR	NCI
	Adriamycin	ADR	Adria Laboratories
	Bleomycin	BLM	Bristol Laboratories
	Mitomycin C	MTC	Bristol Laboratories
Random synthetics and miscellaneous	BCNU	BCNU	NCI
	CCNU	CCNU	NCI
	Methyl-CCNU	MCCNU	NCI
	Streptozotocin	STZ	NCI
	DTIC	DTIC	Dome Laboratories
	Hexamethylmelamine	HXM	NCI
	Dibromodulcitol	DBD	NCI
	Hydroxyurea	HYD	E. R. Squibb & Sons
	Procarbazine	PCB	Roche Laboratories
	L-Asparaginase	L-ASP	NCI
	Dibromomannitol	DBM	NCI
	5-Azacytidine	5-AZC	NCI

TABLE 1.2.—Definitions of activity

Activity	Symbol
Not evaluated	NE
Adequate evaluation, drug inactive	–
Inadequate evaluation, evidence of drug activity but not clearly established	+
Adequate evaluation, drug definitely active	++

priate dose schedule. Similarly, the most reliable definition of response is 50% or greater shrinkage of a measurable lesion. Both of these definitions were most difficult to apply to data for older, now commercially available, agents. With time, the newer agents have undergone better-defined trials employing more carefully defined criteria of response in more disease-oriented trials. However, as the science, art, and practice of chemotherapy have advanced, the new drug trials have been in patients with more advanced disease who have failed to respond to more prior vigorous chemotherapy, including combinations. Thus, we have tended to put more weight on the phase II trials of the newer drugs with regard to efficacy data (a phase II trial attempts to establish evidence of drug activity in a single tumor or many tumor types). There are few phase III trials of the newer drugs as single agents in most tumor types. Instead, most phase III trials involve combination chemotherapy studies with these drugs (a phase III trial is designed to compare a new form of therapy with a standard form of therapy).

The sources from which the data were derived were mostly clinical trials by cooperative groups or single investigators in the United States. The studies were usually of a single drug in a broad phase II trial, including patients with many different types of cancer, or specific disease-oriented studies of a given drug.

Table 5 presents a disease-oriented analysis of the 17 tumors for which a decision on the activity of the 30 drugs is possible on the basis of adequate clinical evaluation. It shows that such a decision on more than half the drugs can be made for only five tumors. These include the three most common tumors (colon, lung, breast), head and neck cancer, and melanoma, an uncommon tumor that has been extensively studied because its lesions are readily accessible to measurement and analysis. Among the other 12 tu-

TABLE 2.—Cross-reference chart of drug-tumor interactions*

Drugs	Tumor types: Bladder	Brain	Breast	Cervix	Colon	Esophagus	Head and neck	Kidney	Lung	Melanoma	Pancreas	Prostate	Ovary	Sarcoma	Stomach	Testicle	Uterus	Totals of tumor activity: NE	–	+	++
Alkylating agents																					
CYC	NE	NE	++	++	+	NE	+	NE	++	–	NE	+	++	++	NE	NE	NE	8	1	3	5
HN2	NE	NE	+	NE	–	NE	–	NE	++	–	NE	+	++	NE	+	NE	NE	9	3	3	2
CHL	NE	NE	+	+	–	NE	+	NE	NE	–	NE	NE	++	+	+	NE	NE	9	2	5	1
MPL	NE	NE	++	+	–	NE	NE	NE	–	–	NE	NE	++	NE	NE	+	NE	10	3	2	2
BUS	NE	NE	NE	NE	NE	NE	NE	NE	–	NE	NE	–	NE	NE	NE	NE	NE	15	2	0	0
Antimetabolites																					
5-FU	+	NE	++	++	++	NE	+	–	–	–	++	+	++	NE	++	NE	NE	5	3	3	6
MTX	NE	+	++	+	+	NE	++	NE	++	–	NE	NE	+	NE	NE	NE	NE	9	1	4	3
6-MP	NE	NE	–	NE	–	NE	+	NE	–	–	NE	NE	NE	NE	NE	NE	NE	12	4	1	0
6-TG	NE	NE	NE	NE	–	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	16	1	0	0
Ara-C	NE	NE	–	NE	–	NE	NE	NE	–	–	NE	NE	NE	NE	NE	NE	NE	13	4	0	0
Mitotic inhibitors																					
VCR	NE	+	++	+	–	NE	NE	NE	+	–	NE	NE	–	++	NE	NE	NE	9	3	3	2
VBL	NE	+	++	NE	–	NE	+	NE	–	–	NE	NE	NE	NE	NE	++	NE	10	3	2	2
Antitumor antibiotics																					
ACT	NE	NE	–	NE	–	NE	NE	NE	NE	+	NE	NE	NE	++	NE	++	NE	12	2	1	2
MTH	NE	–	–	NE	NE	NE	NE	NE	–	–	NE	–	NE	–	NE	++	NE	10	6	0	1
DNR	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	17	0	0	0
ADR	++	NE	++	+	–	NE	+	–	++	–	NE	NE	++	++	+	+	NE	5	3	4	5
BLM	NE	NE	–	+	–	+	++	NE	+	–	NE	NE	NE	NE	NE	++	NE	9	3	3	2
MTC	+	NE	++	NE	++	NE	NE	NE	+	–	+	NE	NE	+	++	NE	NE	9	1	4	3
Random synthetics and miscellaneous																					
BCNU	NE	++	+	NE	+	NE	NE	NE	+	+	–	NE	NE	NE	+	NE	NE	10	1	5	1
CCNU	NE	++	–	NE	–	NE	–	–	++	+	NE	NE	+	NE	–	NE	NE	8	5	2	2
MCCNU	NE	++	–	+	++	NE	+	–	+	+	NE	NE	–	–	–	NE	NE	6	5	4	2
STZ'	NE	NE	–	NE	–	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	15	2	0	0
DTIC	NE	NE	–	NE	–	NE	–	–	–	++	NE	NE	NE	+	NE	NE	NE	10	5	1	1
HXM	NE	NE	+	+	+	NE	+	–	++	–	NE	NE	++	–	NE	NE	NE	8	3	4	2
DBD	NE	NE	+	NE	–	NE	+	+	+	+	NE	NE	NE	+	NE	NE	NE	10	1	6	0
HYD	NE	NE	–	NE	–	NE	+	+	+	–	NE	NE	NE	NE	NE	NE	NE	11	3	3	0
PCB	NE	+	NE	NE	–	NE	–	NE	+	–	NE	NE	NE	NE	NE	NE	NE	12	3	2	0
L-ASP	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	17	0	0	0
DBM	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	17	0	0	0
5-AZC	NE	NE	–	NE	–	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	15	2	0	0
Totals of drug activity																					
NE	27	22	6	20	5	29	14	22	8	7	27	25	19	19	22	24	30				
–	0	1	11	0	18	0	4	6	8	17	1	2	2	3	2	0	0				
+	2	4	5	8	4	1	10	2	8	5	1	3	2	4	4	2	0				
++	1	3	8	2	3	0	2	0	6	1	1	0	7	4	2	4	0				

*See Addendum.

mors, the percentage of drugs inadequately evaluated or completely untested ranges from 63% (sarcoma) to 100% (uterus).

Table 6 ranks the solid tumors by the percentage of drugs having definite activity. Table 7 is a similar ranking but is based on drugs with evidence of activity as well as those with definite activity.

From a drug-oriented standpoint, table 8 ranks

the drugs with respect to the number of tumors in which each has been adequately evaluated. Only 12 drugs have received adequate testing in about half of the tumors. Inadequate testing, or none at all, is quite evident for a large number of agents. Table 9 ranks the drugs by the number of tumors where definite responsiveness has been observed; similarly, table 10 ranks them by definite activity or evidence of activity.

The data presented and our evaluations may be open to many questions and criticisms. However, it is the most defined data available and does permit some comparisons. We have specifically elected not to draw any conclusions here, as these have been and will continue to be presented elsewhere.

It would be difficult to reference all of the sources for these data, so we have provided a general reference list which includes most of the pertinent reports or will lead the inquisitive reader to them (2-33). Obviously, with 510 pieces of data (30 drugs × 17 tumors) some errors are possible and for these we apologize to the reader. Also, an effort has been made to have the data reflect as current a level of knowledge as possible (*see* Addendum).

DISEASE-ORIENTED VIEW OF DRUG ACTIVITY

It would be very difficult to detail the nuances of the data and our evaluation for each of the 30 drugs and 17 tumors. As an adjunct to the disease-oriented tables (tables 3.1-3.21), the following short explanatory paragraphs may provide better understanding of the data and its evaluation.

Bladder Cancer (Table 3.1)

The evaluation of 5-fluorouracil is based on cumulative data from 16 different studies in which response varied from 0 to 75%. No study includes more than 12 patients. Several adjuvant studies with 5-fluorouracil have shown no survival benefit although the studies were not well controlled.

Brain Tumors (Table 3.2)

The evaluation of mithramycin as an inactive drug is based on a Brain Tumor Study Group protocol (Excerpta Medica International Congress Series No. 193, p 33, Sept 20, 1969) where there was no difference in survival compared to a control group.

Methotrexate, vincristine, vinblastine, and procarbazine were classified as "+" because of:

a. The small number of evaluable patients,
b. The lack of disease-oriented study, and
c. The fact that most of the data on these drugs are from older and broader phase II studies where the response criteria were not well defined.

Bleomycin is nonevaluable since the data are from Japanese studies, where response criteria are poorly defined. (*See* Addendum)

Colon Cancer (Table 3.5)

Of the alkylating agents, only cyclophosphamide has shown some consistent hints of activity in both old and more recent studies. Responses are real but brief (3-9 weeks) using the high intermittent dose schedule. Melphalan has been ranked inactive because of the less reliable quality of the data. Methotrexate is the only antimetabolite showing some hint of activity and is now undergoing confirmatory study. The nitrosoureas show a low order of activity with somewhat more activity in previously untreated patients. Methyl-CCNU is felt to be more active because of its increasingly established role in combinations. Hexamethylmelamine has shown some activity and should be evaluated more extensively.

Head and Neck Cancer (Table 3.7)

Data are given for all histologic types and locations. The methotrexate data include an overall summation for all routes and dose levels.

Melanoma (Table 3.13)

Although the response rates for vincristine, vinblastine, cyclophosphamide, and hydroxyurea range from 12% to 23%, the data available from studies employing strict criteria for response yield the following results: cyclophosphamide (5%), hydroxyurea (8%), vinblastine (13%), and vincristine (15%). Actinomycin D has never been vigorously evaluated, but some activity has been described in early studies that need further evaluation.

Pancreatic Cancer (Table 3.15)

Only three drugs have been adequately evaluated. Mitomycin C has been ranked below 5-fluorouracil because of the less extensive evaluation, with the bulk of the data coming from early studies where response criteria were vague.

Sarcoma (Table 3.18)

The evaluation of adriamycin as an active drug is based on its being the most studied agent in this tumor. It has consistent activity in many studies involving patients who failed to respond to the other three active drugs (cyclophosphamide, vincristine, and actinomycin D). It also has shown superior activity in combinations with other agents.

Stomach Cancer (Table 3.19)

Mechlorethamine and chlorambucil have shown hints of activity, with the data from the latter being somewhat more reliable than those from the former. Alkylating agent activity must be evaluated. Differential activity within the nitrosoureas is seen for BCNU with good data from a single Mayo Clinic study. Adriamycin has recently shown some activity in specific disease-oriented evaluations conducted by Japanese investigators as well as at the Mayo Clinic.

Table 3.1.--Drug activity in bladder cancer

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
ADR	++	27/97	28
5-FU *	+	26/74	35
MTC	+	13/51	25
None	-		
CYC	NE	2/10	-
HN2	NE	-	-
CHL	NE	0/10	-
MPL	NE	-	-
BUS	NE	-	-
MTX	NE	4/11	-
6-MP	NE	-	-
6-TG	NE	-	-
Ara-C	NE	-	-
VCR	NE	-	-
VBL	NE	-	-
ACT	NE	-	-
MTH	NE	-	-
DNR	NE	-	-
BLM	NE	-	-
BCNU	NE	-	-
CCNU	NE	-	-
MCCNU	NE	-	-
STZ	NE	-	-
DTIC	NE	0/7	-
HXM	NE	3/10	-
DBD	NE	0/4	-
HYD	NE	5/8	-
PCB	NE	1/3	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	-	-

Table 3.2.--Drug activity in brain cancer

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
BCNU	++	67/142	47
CCNU	++	47/115	41
MCCNU	++	13/40	33
MTX*	+	35/65	54
VCR *	+	33/58	57
VBL *	+	21/48	44
PCB *	+	14/27	52
MTH*	-	21/41	52
CYC	NE	0/14	-
HN2	NE	-	-
CHL	NE	-	-
MPL	NE	-	-
BUS	NE	-	-
5-FU	NE	1/19	5
6-MP	NE	-	-
6-TG	NE	-	-
Ara-C	NE	-	-
ACT	NE	-	-
DNR	NE	-	-
ADR	NE	-	-
BLM *	NE	15/21	71
MTC	NE	-	-
STZ	NE	-	-
DTIC	NE	2/5	-
HXM	NE	-	-
DBD	NE	-	-
HYD	NE	9/10	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	1/1	-

Table 3.3.--Drug activity in breast cancer

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
CYC	++	182/529	34
MPL	++	20/86	23
5-FU	++	324/1263	26
MTX	++	120/356	34
VCR	++	47/226	20
VBL	++	19/95	20
ADR	++	67/193	35
MTC	++	41/110	37
HN2	+	32/92	35
CHL	+	11/54	20
BCNU	+	16/76	21
HXM	+	11/54	20
DBD	+	20/53	37
6-MP	-	6/45	13
Ara-C	-	6/64	9
ACT	-	5/44	11
MTH	-	14/70	20
BLM	-	3/26	12
CCNU	-	20/171	12
MCCNU	-	3/95	3
STZ	-	2/19	11
DTIC	-	5/51	10
HYD	-	4/21	19
5-AZC	-	11/53	21
BUS	NE	0/8	-
6-TG	NE	-	-
DNR	NE	0/2	-
PCB	NE	1/21	5
L-ASP	NE	0/1	-
DBM	NE	-	-

Table 3.4.--Drug activity in cervical cancer

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
CYC	++	36/187	19
5-FU	++	37/159	23
CHL	+	11/44	25
MPL	+	4/20	20
MTX	+	5/24	21
VCR	+	10/34	29
ADR	+	9/28	32
BLM	+	8/78	10
MCCNU	+	4/24	17
HXM	+	11/40	28
None	-		
HN2	NE	0/12	-
BUS	NE	-	-
6-MP	NE	-	-
6-TG	NE	-	-
Ara-C	NE	-	-
VBL	NE	-	-
ACT	NE	-	-
MTH	NE	-	-
DNR	NE	-	-
MTC	NE	4/18	22
BCNU	NE	1/7	-
CCNU	NE	1/5	-
STZ	NE	-	-
DTIC	NE	3/12	-
DBD	NE	-	-
HYD	NE	2/13	-
PCB	NE	-	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	-	-

Table 3.5.--Drug activity in colon cancer

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
5-FU	++	454/2107	21
MTC	++	35/218	16
MCCNU *	++	18/148	12
CYC *	+	15/71	21
MTX *	+	19/111	17
BCNU	+	17/128	13
HXM *	+	11/95	12
HN2	-	6/50	12
CHL	-	5/55	9
MPL *	-	19/110	17
6-MP	-	2/50	4
6-TG	-	1/35	3
Ara-C	-	13/137	10
VCR	-	0/26	-
VBL	-	4/64	5
ACT	-	7/48	15
ADR	-	8/92	9
BLM	-	0/23	-
CCNU	-	20/222	9
STZ	-	6/65	9
DTIC	-	7/84	8
DBD	-	4/42	10
HYD	-	16/151	10
PCB	-	0/35	-
5-AZC	-	5/52	10
BUS	NE	1/10	-
MTH	NE	4/28	14
DNR	NE	0/9	-
L-ASP	NE	0/6	-
DBM	NE	-	-

Table 3.6.--Drug activity in esophageal cancer

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
BLM	+	7/42	17
None	-		
CYC	NE	-	-
HN2	NE	-	-
CHL	NE	-	-
MPL	NE	-	-
BUS	NE	-	-
5-FU	NE	3/18	17
MTX	NE	-	-
6-MP	NE	-	-
6-TG	NE	-	-
Ara-C	NE	-	-
VCR	NE	-	-
VBL	NE	-	-
ACT	NE	-	-
MTH	NE	-	-
DNR	NE	-	-
ADR	NE	-	-
MTC	NE	-	-
BCNU	NE	-	-
CCNU	NE	2/19	10
MCCNU	NE	-	-
STZ	NE	-	-
DTIC	NE	0/3	-
HXM	NE	1/3	-
DBD	NE	0/1	-
HYD	NE	-	-
PCB	NE	-	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	-	-

Table 3.7.--Drug activity in head and neck cancer (all types)

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
MTX *	++	298/630	47
BLM	++	24/158	15
CYC	+	28/77	36
CHL	+	5/34	15
5-FU	+	18/118	15
6-MP	+	6/45	12
VBL	+	10/35	29
ADR	+	13/67	19
MCCNU	+	6/40	15
HXM	+	9/75	12
DBD	+	11/50	22
HYD	+	7/18	39
HN2	-	5/66	8
CCNU	-	4/50	8
DTIC	-	1/24	5
PCB	-	3/31	10
MPL	NE	-	-
BUS	NE	-	-
6-TG	NE	-	-
Ara-C	NE	-	-
VCR	NE	-	-
ACT	NE	-	-
MTH	NE	-	-
DNR	NE	-	-
MTC	NE	2/11	-
BCNU	NE	4/25	16
STZ	NE	2/7	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	1/5	-

Table 3.8.--Drug activity in lung cancer (all cell types)

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
CYC	++	303/1513	20
HN2	++	447/1442	31
MTX	++	92/416	22
ADR	++	53/314	17
CCNU	++	46/262	18
HXM	++	94/524	18
MTC	+	47/203	23
BCNU	+	10/91	11
MCCNU	+	29/289	10
DBD	+	37/255	15
HYD	+	14/84	17
PCB	+	31/166	19
VCR	+	6/43	14
BLM	+	40/331	12
BUS	-	15/169	9
5-FU	-	11/157	7
6-MP	-	3/105	3
Ara-C	-	0/43	-
VBL	-	17/239	7
MTH	-	4/46	9
DTIC	-	15/132	11
MPL	-	5/67	7
CHL	NE	1/21	5
6-TG	NE	-	-
ACT	NE	0/16	-
DNR	NE	1/14	-
STZ	NE	2/5	-
L-ASP	NE	0/1	-
DBM	NE	-	-
5-AZC	NE	0/9	-

Table 3.9.--Drug activity in lung cancer (squamous cell type)

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
CYC	++	46/232	20
HN2	++	38/183	21
MTX	++	35/140	25
DBD	++	9/39	23
ADR	+	13/70	19
BLM	+	34/265	13
CCNU	+	10/58	17
MCCNU	+	13/80	16
HXM	+	11/99	11
PCB	+	7/55	13
BUS	-	4/61	7
MTC	-	0/22	-
BCNU	-	1/38	3
CHL	NE	-	-
MPL	NE	-	-
5-FU	NE	-	-
6-MP	NE	0/16	-
6-TG	NE	-	-
Ara-C	NE	-	-
VCR	NE	-	-
VBL	NE	3/20	16
ACT	NE	-	-
MTH	NE	-	-
DNR	NE	-	-
STZ	NE	-	-
DTIC	NE	2/11	-
HYD	NE	-	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	-	-

Table 3.10.--Drug activity in lung cancer (adenocarcinoma)

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
CYC	++	10/48	21
HN2	++	23/80	29
MTX	+	8/25	32
ADR	+	8/53	15
BLM	+	4/30	13
CCNU	+	9/54	17
HXM	+	11/59	19
BUS	-	1/28	4
MCCNU	-	4/67	6
CHL	NE	-	-
MPL	NE	-	-
5-FU	NE	-	-
6-MP	NE	-	-
6-TG	NE	-	-
Ara-C	NE	-	-
VCR	NE	-	-
VBL	NE	1/6	-
ACT	NE	-	-
MTH	NE	-	-
DNR	NE	-	-
MTC	NE	3/11	-
BCNU	NE	3/15	20
STZ	NE	-	-
DTIC	NE	0/14	-
DBD	NE	1/12	-
HYD	NE	-	-
PCB	NE	3/16	19
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	-	-

Table 3.11.--Drug activity in lung cancer (small cell type)

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
CYC	++	92/296	31
HN2	++	31/80	39
MTX	++	31/79	39
HXM	++	24/67	36
PCB	++	11/44	25
ADR	+	12/48	25
BCNU	+	4/19	21
CCNU	+	7/48	15
MCCNU	+	4/33	12
DBD	+	3/15	20
BLM	-	0/27	-
CHL	NE	-	-
MPL	NE	-	-
BUS	NE	3/17	18
5-FU	NE	-	-
6-MP	NE	1/9	-
6-TG	NE	-	-
Ara-C	NE	-	-
VCR	NE	2/6	-
VBL	NE	3/9	-
ACT	NE	-	-
MTH	NE	-	-
DNR	NE	-	-
MTC	NE	1/11	-
STZ	NE	-	-
DTIC	NE	-	-
HYD	NE	-	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	-	-

Table 3.12.--Drug activity in lung cancer (large cell type)

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
HN2	++	38/148	26
CYC	+	5/22	23
MTX	+	12/104	12
ADR	+	7/27	26
CCNU	+	9/37	24
MCCNU	+	6/30	20
HXM	+	6/37	17
PCB	+	6/17	35
6-MP	-	1/29	3
CHL	NE	-	-
MPL	NE	-	-
BUS	NE	1/6	-
5-FU	NE	-	-
6-TG	NE	-	-
Ara-C	NE	-	-
VCR	NE	-	-
VBL	NE	2/2	-
ACT	NE	-	-
MTH	NE	-	-
DNR	NE	-	-
BLM	NE	0/2	-
MTC	NE	1/2	-
BCNU	NE	2/20	9
STZ	NE	-	-
DTIC	NE	-	-
DBD	NE	-	-
HYD	NE	-	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	-	-

Table 3.13.--Drug activity in melanoma

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
DTIC	++	208/853	24
ACT*	+	19/55	35
BCNU	+	22/122	18
CCNU	+	17/133	13
MCCNU	+	18/101	18
DBD	+	8/42	19
CYC*	-	9/55	16
HN2	-	3/45	7
CHL	-	2/22	9
MPL	-	8/52	15
5-FU	-	1/43	2
MTX	-	2/25	8
6-MP	-	2/47	4
Ara-C	-	1/52	2
VCR*	-	6/52	12
VBL*	-	11/71	15
MTH	-	9/79	11
ADR	-	0/19	-
BLM	-	0/34	-
MTC	-	9/65	14
HXM	-	2/42	5
HYD*	-	29/127	23
PCB	-	8/50	16
BUS	NE	1/10	-
6-TG	NE	-	-
DNR	NE	0/6	-
STZ	NE	0/3	-
L-ASP	NE	1/5	-
DBM	NE	-	-
5-AZC	NE	3/16	19

Table 3.14.--Drug activity in ovarian cancer

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
CYC	++	115/262	44
CHL	++	200/390	51
MPL	++	235/494	47
5-FU	++	32/101	32
ADR	++	18/48	38
HXM	++	21/54	39
HN2	++	25/81	31
CCNU	+	7/22	32
MTX	+	4/16	25
VCR	-	0/17	-
MCCNU	-	0/18	-
BUS	NE	-	-
6-MP	NE	-	-
6-TG	NE	-	-
Ara-C	NE	-	-
VBL	NE	3/20	15
ACT	NE	-	-
MTH	NE	-	-
DNR	NE	-	-
BLM	NE	0/12	-
MTC	NE	-	-
BCNU	NE	2/34	6
STZ	NE	0/9	-
DTIC	NE	0/9	-
DBD	NE	1/8	-
HYD	NE	-	-
PCB	NE	-	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	1/4	-

Table 3.15.--Drug activity in pancreatic cancer

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
5-FU *	++	60/212	28
MTC *	+	12/44	27
BCNU	-	0/37	-
CYC	NE	1/5	-
HN2	NE	1/7	-
MPL	NE	-	-
CHL	NE	4/6	-
BUS	NE	-	-
MTX	NE	-	-
6-MP	NE	-	-
6-TG	NE	-	-
Ara-C	NE	-	-
VCR	NE	-	-
VBL	NE	-	-
ACT	NE	0/4	-
MTH	NE	0/4	-
DNR	NE	-	-
ADR	NE	-	-
BLM	NE	-	-
CCNU	NE	3/19	16
MCCNU	NE	0/13	-
STZ	NE	3/27	11
DTIC	NE	0/10	-
HXM	NE	0/6	-
DBD	NE	-	-
HYD	NE	-	-
PCB	NE	-	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	1/2	-

Table 3.16.--Drug activity in prostatic cancer

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
5-FU	+	19/66	29
CYC	+	8/57	14
HN2	+	12/31	39
BUS	-	1/16	6
MTH	-	2/36	6
CHL	NE	-	-
MPL	NE	-	-
MTX	NE	1/3	-
6-MP	NE	-	-
6-TG	NE	-	-
Ara-C	NE	-	-
VCR	NE	2/22	9
VBL	NE	1/2	-
ACT	NE	-	-
DNR	NE	-	-
ADR	NE	2/9	-
BLM	NE	-	-
MTC	NE	1/8	-
BCNU	NE	2/15	14
CCNU	NE	-	-
MCCNU	NE	2/19	11
STZ	NE	-	-
DTIC	NE	0/6	-
HXM	NE	2/6	-
DBD	NE	1/3	-
HYD	NE	-	-
PCB	NE	-	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	-	-

Table 3.17.--Drug activity in renal cancer

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
DBD	+	4/19	21
HYD	+	7/25	28
5-FU	-	3/40	8
ADR	-	4/56	7
CCNU	-	4/59	7
MCCNU	-	0/21	-
DTIC	-	1/29	3
HXM	-	1/17	6
CYC	NE	2/12	-
HN2	NE	-	-
CHL	NE	2/15	13
MPL	NE	-	-
BUS	NE	-	-
MTX	NE	-	-
6-MP	NE	1/15	7
6-TG	NE	-	-
Ara-C	NE	-	-
VCR	NE	-	-
VBL	NE	1/12	-
ACT	NE	-	-
MTH	NE	-	-
DNR	NE	-	-
BLM	NE	1/7	-
MTC	NE	4/36	11
BCNU	NE	-	-
STZ	NE	0/10	-
PCB	NE	-	-
L-ASP	NE	0/1	-
DBM	NE	-	-
5-AZC	NE	0/2	-

Table 3.19.--Drug activity in stomach cancer

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
5-FU	++	101/448	23
MTC	++	63/211	30
HN2*	+	4/25	16
CHL*	+	3/18	17
ADR*	+	7/27	26
BCNU	+	6/33	18
CCNU	-	1/35	3
MCCNU	-	1/28	4
CYC	NE	0/4	-
MPL	NE	-	-
BUS	NE	-	-
MTX	NE	0/2	-
6-MP	NE	-	-
6-TG	NE	-	-
Ara-C	NE	3/11	-
VCR	NE	-	-
VBL	NE	-	-
ACT	NE	-	-
MTH	NE	3/11	-
DNR	NE	-	-
BLM	NE	-	-
STZ	NE	0/4	-
DTIC	NE	2/15	13
HXM	NE	2/12	-
DBD	NE	3/8	-
HYD	NE	6/31	19
PCB	NE	-	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	0/3	-

Table 3.18.--Drug activity in sarcoma

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
CYC	++	48/93	52
VCR	++	25/53	47
ACT	++	20/83	24
ADR*	++	88/351	25
CHL	+	7/28	25
MTC	+	10/33	33
DTIC	+	15/127	12
DBD	+	7/44	16
MTH	-	2/39	5
MCCNU	-	4/72	6
HXM	-	1/43	2
HN2	NE	-	-
MPL	NE	-	-
BUS	NE	-	-
5-FU	NE	-	-
MTX	NE	-	-
6-MP	NE	-	-
6-TG	NE	-	-
Ara-C	NE	-	-
VBL	NE	-	-
DNR	NE	-	-
BLM	NE	-	-
BCNU	NE	4/17	24
CCNU	NE	1/22	5
STZ	NE	1/7	-
HYD	NE	-	-
PCB	NE	-	-
L-ASP	NE	0/1	-
DBM	NE	-	-
5-AZC	NE	0/4	-

Table 3.20.--Drug activity in testicular cancer

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
VBL	++	17/25	52
ACT	++	16/31	52
MTH	++	155/431	36
BLM	++	24/57	42
MPL	+	49/86	57
ADR	+	12/60	20
None	-		
CYC	NE	2/2	-
HN2	NE	-	-
CHL	NE	6/8	-
BUS	NE	-	-
5-FU	NE	4/15	-
MTX	NE	4/10	-
6-MP	NE	-	-
6-TG	NE	-	-
Ara-C	NE	-	-
VCR	NE	4/7	-
DNR	NE	-	-
MTC	NE	-	-
BCNU	NE	2/8	-
CCNU	NE	-	-
MCCNU	NE	0/4	-
STZ	NE	0/2	-
DTIC	NE	0/4	-
HXM	NE	2/6	-
DBD	NE	0/1	-
HYD	NE	2/6	-
PCB	NE	1/3	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	0/2	-

Table 3.21.--Drug activity in uterine cancer

Drugs	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
None	+		
None	-		
CYC	NE	4/16	25
HN2	NE	-	-
CHL	NE	0/11	-
MPL	NE	-	-
BUS	NE	-	-
5-FU	NE	7/34	20
MTX	NE	-	-
6-MP	NE	-	-
6-TG	NE	-	-
Ara-C	NE	-	-
VCR	NE	-	-
VBL	NE	-	-
ACT	NE	-	-
MTH	NE	-	-
DNR	NE	-	-
ADR	NE	2/6	-
BLM	NE	-	-
MTC	NE	-	-
BCNU	NE	-	-
CCNU	NE	-	-
MCCNU	NE	-	-
STZ	NE	-	-
DTIC	NE	-	-
HXM	NE	2/8	-
DBD	NE	-	-
HYD	NE	-	-
PCB	NE	-	-
L-ASP	NE	-	-
DBM	NE	-	-
5-AZC	NE	-	-

NOTE: Drugs marked with an * are discussed in the text under "Disease-Oriented View of Drug Activity."

Table 4.1.--Activity of ACT

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Testicle	++	16/31	52
Sarcoma	++	20/83	24
Melanoma	+	19/55	35
Colon	-	7/48	15
Breast	-	5/44	11
Lung	NE	0/16	-
Pancreas	NE	0/4	-
Ovary	NE	-	-
Prostate	NE	-	-
Stomach	NE	-	-
Cervix	NE	-	-
Head and Neck	NE	-	-
Bladder	NE	-	-
Kidney	NE	-	-
Esophagus	NE	-	-
Brain	NE	-	-
Uterus	NE	-	-

Table 4.2.--Activity of ADR

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Lung	++	53/314	17
Breast	++	67/193	35
Ovary	++	18/48	38
Bladder	++	27/97	28
Sarcoma	++	88/351	25
Stomach	+	7/27	26
Cervix	+	9/28	32
Head and Neck	+	13/67	19
Testicle	+	12/60	20
Colon	-	8/92	9
Kidney	-	4/56	7
Melanoma	-	0/19	-
Pancreas	NE	-	-
Prostate	NE	2/9	-
Esophagus	NE	-	-
Brain	NE	-	-
Uterus	NE	2/6	-

Table 4.3.--Activity of Ara-C

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
None	+		
Colon	-	13/137	10
Lung	-	0/43	-
Breast	-	6/64	9
Melanoma	-	1/52	2
Pancreas	NE	-	-
Ovary	NE	-	-
Prostate	NE	-	-
Stomach	NE	3/11	-
Cervix	NE	-	-
Head and Neck	NE	-	-
Bladder	NE	-	-
Kidney	NE	-	-
Esophagus	NE	-	-
Brain	NE	-	-
Testicle	NE	-	-
Sarcoma	NE	-	-
Uterus	NE	-	-

Table 4.4.--Activity of L-ASP

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
None	+		
None	-		
Colon	NE	0/6	-
Lung	NE	0/1	-
Breast	NE	0/1	-
Pancreas	NE	-	-
Ovary	NE	-	-
Prostate	NE	-	-
Stomach	NE	-	-
Cervix	NE	-	-
Head and Neck	NE	-	-
Bladder	NE	-	-
Kidney	NE	0/1	-
Esophagus	NE	-	-
Brain	NE	-	-
Testicle	NE	-	-
Melanoma	NE	1/5	-
Sarcoma	NE	0/1	-
Uterus	NE	-	-

Table 4. 5. --Activity of 5-AZC

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
None	+		
Colon	-	5/52	10
Breast	-	11/53	21
Lung	NE	0/9	-
Pancreas	NE	1/2	-
Ovary	NE	1/4	-
Prostate	NE	-	-
Stomach	NE	0/3	-
Cervix	NE	-	-
Head and Neck	NE	1/5	-
Bladder	NE	-	-
Kidney	NE	0/2	-
Esophagus	NE	-	-
Brain	NE	1/1	-
Testicle	NE	0/2	-
Melanoma	NE	3/16	19
Sarcoma	NE	0/4	-
Uterus	NE	-	-

Table 4. 6. --Activity of BCNU

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Brain	++	67/142	47
Colon	+	17/128	13
Lung	+	10/91	11
Breast	+	16/76	21
Stomach	+	6/33	18
Melanoma	+	22/122	18
Pancreas	-	0/37	-
Ovary	NE	2/34	6
Prostate	NE	2/15	14
Cervix	NE	1/7	-
Head and Neck	NE	4/25	16
Bladder	NE	-	-
Kidney	NE	-	-
Esophagus	NE	-	-
Testicle	NE	2/8	-
Sarcoma	NE	4/17	24
Uterus	NE	-	-

Table 4. 7. --Activity of BLM

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Head and Neck	++	24/158	15
Testicle	++	24/57	42
Cervix	+	8/78	10
Esophagus	+	7/42	17
Lung	+	40/331	12
Colon	-	0/23	-
Breast	-	3/26	12
Melanoma	-	0/34	-
Pancreas	NE	-	-
Ovary	NE	0/12	-
Prostate	NE	-	-
Stomach	NE	-	-
Bladder	NE	-	-
Kidney	NE	1/7	-
Brain	NE	15/21	71
Sarcoma	NE	-	-
Uterus	NE	-	-

Table 4. 8. --Activity of BUS

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
None	+		
Lung	-	15/169	9
Prostate	-	1/16	6
Colon	NE	1/10	-
Breast	NE	0/8	-
Pancreas	NE	-	-
Ovary	NE	-	-
Stomach	NE	-	-
Cervix	NE	-	-
Head and Neck	NE	-	-
Bladder	NE	-	-
Kidney	NE	-	-
Esophagus	NE	-	-
Brain	NE	-	-
Testicle	NE	-	-
Melanoma	NE	1/10	-
Sarcoma	NE	-	-
Uterus	NE	-	-

Table 4. 9. --Activity of CCNU

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Lung	++	46/262	18
Brain	++	47/115	41
Ovary	+	7/22	32
Melanoma	+	17/133	13
Colon	-	20/222	9
Breast	-	20/171	12
Stomach	-	1/35	3
Head and Neck	-	4/50	8
Kidney	-	4/59	7
Pancreas	NE	3/19	16
Prostate	NE	-	-
Cervix	NE	1/5	-
Bladder	NE	-	-
Esophagus	NE	2/19	10
Testicle	NE	-	-
Sarcoma	NE	1/22	5
Uterus	NE	-	-

Table 4. 10. --Activity of CHL

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Ovary	++	200/390	51
Breast	+	11/54	20
Stomach	+	3/18	17
Cervix	+	11/44	25
Head and Neck	+	5/34	15
Sarcoma	+	7/28	25
Colon	-	5/55	9
Melanoma	-	2/22	9
Lung	NE	1/21	5
Pancreas	NE	4/6	-
Prostate	NE	-	-
Bladder	NE	0/10	-
Kidney	NE	2/15	13
Esophagus	NE	-	-
Brain	NE	-	-
Testicle	NE	6/8	-
Uterus	NE	0/11	-

Table 4. 11. --Activity of CYC

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Lung	++	303/1513	20
Breast	++	182/529	34
Ovary	++	115/262	44
Cervix	++	36/187	19
Sarcoma	++	48/93	52
Colon	+	15/71	21
Head and Neck	+	28/77	36
Prostate	+	8/57	14
Melanoma	-	9/55	16
Pancreas	NE	1/5	-
Stomach	NE	0/4	-
Bladder	NE	2/10	-
Kidney	NE	2/12	-
Esophagus	NE	-	-
Brain	NE	0/14	-
Testicle	NE	2/2	-
Uterus	NE	4/16	25

Table 4. 12. --Activity of DNR

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
None	+		
None	-		
Colon	NE	0/9	-
Lung	NE	1/14	-
Breast	NE	0/2	-
Pancreas	NE	-	-
Ovary	NE	-	-
Prostate	NE	-	-
Stomach	NE	-	-
Cervix	NE	-	-
Head and Neck	NE	-	-
Bladder	NE	-	-
Kidney	NE	-	-
Esophagus	NE	-	-
Brain	NE	-	-
Testicle	NE	-	-
Melanoma	NE	0/6	-
Sarcoma	NE	-	-
Uterus	NE	-	-

Table 4.13.--Activity of DBD

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
Lung	+	37/255	15
Breast	+	20/53	37
Head and Neck	+	11/50	22
Kidney	+	4/19	21
Melanoma	+	8/42	19
Sarcoma	+	7/44	16
Colon	-	4/42	10
Pancreas	NE	-	-
Ovary	NE	1/8	-
Prostate	NE	1/3	-
Stomach	NE	3/8	-
Cervix	NE	-	-
Bladder	NE	0/4	-
Esophagus	NE	0/1	-
Brain	NE	-	-
Testicle	NE	0/1	-
Uterus	NE	-	-

Table 4.14.--Activity of DBM

Tumor	Activity	No. of responses/ No. of evaluable patients	Responses rate (%)
None	++		
None	+		
None	-		
Colon	NE		-
Lung	NE	-	-
Breast	NE	-	-
Pancreas	NE	-	-
Ovary	NE	-	-
Prostate	NE	-	-
Stomach	NE	-	-
Cervix	NE	-	-
Head and Neck	NE	-	-
Bladder	NE	-	-
Kidney	NE	-	-
Esophagus	NE	-	-
Brain	NE	-	-
Testicle	NE	-	-
Melanoma	NE	-	-
Sarcoma	NE	-	-
Uterus	NE	-	-

Table 4.15.--Activity of DTIC

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Melanoma	++	208/853	24
Sarcoma	+	15/127	12
Colon	-	7/84	8
Lung	-	15/132	11
Breast	-	5/51	10
Head and Neck	-	1/24	5
Kidney	-	1/29	3
Pancreas	NE	0/10	-
Ovary	NE	0/9	-
Prostate	NE	0/6	-
Stomach	NE	2/15	13
Cervix	NE	3/12	-
Bladder	NE	0/7	-
Esophagus	NE	0/3	-
Brain	NE	2/5	-
Testicle	NE	0/4	-
Uterus	NE	-	-

Table 4.16.--Activity of 5-FU

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Colon	++	454/2107	21
Breast	++	324/1263	26
Pancreas	++	60/212	28
Ovary	++	32/101	32
Stomach	++	101/448	23
Cervix	++	37/159	23
Prostate	+	19/66	29
Head and Neck	+	18/118	15
Bladder	+	26/74	35
Lung	-	11/157	7
Kidney	-	3/40	8
Melanoma	-	1/43	2
Esophagus	NE	3/18	17
Brain	NE	1/19	5
Testicle	NE	4/15	27
Sarcoma	NE	-	-
Uterus	NE	7/34	20

Table 4.17.--Activity of HXM

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Lung	++	94/524	18
Ovary	++	21/54	39
Colon	+	11/95	12
Breast	+	11/54	20
Head and Neck	+	9/75	12
Cervix	+	11/40	28
Kidney	-	1/17	6
Melanoma	-	2/42	5
Sarcoma	-	1/43	2
Pancreas	NE	0/6	-
Prostate	NE	2/6	-
Stomach	NE	2/12	-
Bladder	NE	3/10	-
Esophagus	NE	1/3	-
Brain	NE	-	-
Testicle	NE	2/6	-
Uterus	NE	2/8	-

Table 4.18.--Activity of HYD

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
Lung	+	14/84	17
Head and Neck	+	7/18	39
Kidney	+	7/25	28
Colon	-	16/151	10
Breast	-	4/21	19
Melanoma	-	29/127	23
Pancreas	NE	-	-
Ovary	NE	-	-
Prostate	NE	-	-
Stomach	NE	6/31	19
Cervix	NE	2/13	-
Bladder	NE	5/8	-
Esophagus	NE	-	-
Brain	NE	9/10	-
Testicle	NE	2/6	-
Sarcoma	NE	-	-
Uterus	NE	-	-

Table 4.19.--Activity of MPL

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Breast	++	20/86	23
Ovary	++	235/494	47
Cervix	+	4/20	20
Testicle	+	49/86	57
Colon	-	19/110	17
Melanoma	-	8/52	15
Lung	-	5/67	7
Pancreas	NE	-	-
Prostate	NE	-	-
Stomach	NE	-	-
Head and Neck	NE	-	-
Bladder	NE	-	-
Kidney	NE	-	-
Esophagus	NE	-	-
Brain	NE	-	-
Sarcoma	NE	-	-
Uterus	NE	-	-

Table 4.20.--Activity of 6-MP

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
Head and Neck	+	6/45	12
Colon	-	2/50	4
Lung	-	3/105	3
Breast	-	6/45	13
Melanoma	-	2/47	4
Pancreas	NE	-	-
Ovary	NE	-	-
Prostate	NE	-	-
Stomach	NE	-	-
Cervix	NE	-	-
Bladder	NE	-	-
Kidney	NE	1/15	7
Esophagus	NE	-	-
Brain	NE	-	-
Testicle	NE	-	-
Sarcoma	NE	-	-
Uterus	NE	-	-

Table 4. 21. --Activity of MTX

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Lung	++	92/416	22
Breast	++	120/356	34
Head and Neck	++	298/630	47
Colon	+	19/111	17
Cervix	+	5/24	21
Brain	+	35/65	54
Ovary	+	4/16	25
Melanoma	-	2/25	8
Pancreas	NE	-	-
Prostate	NE	1/3	-
Stomach	NE	0/2	-
Bladder	NE	4/11	-
Kidney	NE	-	-
Esophagus	NE	-	-
Testicle	NE	4/10	-
Sarcoma	NE	-	-
Uterus	NE	-	-

Table 4. 22. --Activity of MCCNU

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Brain	++	13/40	30
Colon	++	18/148	12
Lung	+	29/289	10
Cervix	+	4/24	17
Head and Neck	+	6/40	15
Melanoma	+	18/101	18
Breast	-	3/95	3
Ovary	-	0/18	-
Stomach	-	1/28	4
Kidney	-	0/21	-
Sarcoma	-	4/72	6
Pancreas	NE	0/13	-
Prostate	NE	2/19	11
Bladder	NE	-	-
Esophagus	NE	-	-
Testicle	NE	0/4	-
Uterus	NE	-	-

Table 4. 23. --Activity of MTH

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Testicle	++	155/431	36
None	+		
Lung	-	4/46	9
Breast	-	14/70	20
Brain	-	21/41	52
Melanoma	-	9/79	11
Sarcoma	-	2/39	5
Prostate	-	2/36	6
Colon	NE	4/28	14
Pancreas	NE	0/4	-
Ovary	NE	-	-
Stomach	NE	3/11	-
Cervix	NE	-	-
Head and Neck	NE	-	-
Bladder	NE	-	-
Kidney	NE	-	-
Esophagus	NE	-	-
Uterus	NE	-	-

Table 4. 24. --Activity of MTC

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Colon	++	35/218	16
Breast	++	41/110	37
Stomach	++	63/211	30
Lung	+	47/203	23
Pancreas	+	12/44	27
Sarcoma	+	10/33	33
Bladder	+	13/51	25
Melanoma	-	9/65	14
Ovary	NE	-	-
Prostate	NE	1/8	-
Cervix	NE	4/18	22
Head and Neck	NE	2/11	-
Kidney	NE	4/36	11
Esophagus	NE	-	-
Brain	NE	-	-
Testicle	NE	-	-
Uterus	NE	-	-

Table 4. 25. --Activity of HN2

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Lung	++	447/1442	31
Ovary	++	25/81	31
Breast	+	32/92	35
Stomach	+	4/25	16
Prostate	+	12/31	39
Colon	-	6/50	12
Head and Neck	-	5/66	8
Melanoma	-	3/45	7
Pancreas	NE	1/7	-
Cervix	NE	0/12	-
Bladder	NE	-	-
Kidney	NE	-	-
Esophagus	NE	-	-
Brain	NE	-	-
Testicle	NE	-	-
Sarcoma	NE	-	-
Uterus	NE	-	-

Table 4. 26. --Activity of PCB

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
Lung	+	31/166	19
Brain	+	14/27	52
Colon	-	0/35	-
Head and Neck	-	3/31	10
Melanoma	-	8/50	16
Breast	NE	1/21	5
Pancreas	NE	-	-
Ovary	NE	-	-
Prostate	NE	-	-
Stomach	NE	-	-
Cervix	NE	-	-
Bladder	NE	1/3	-
Kidney	NE	-	-
Esophagus	NE	-	-
Testicle	NE	1/3	-
Sarcoma	NE	-	-
Uterus	NE	-	-

Table 4. 27. --Activity of STZ

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
None	+		
Colon	-	6/65	9
Breast	-	2/19	11
Lung	NE	2/5	-
Pancreas	NE	3/27	11
Ovary	NE	0/9	-
Prostate	NE	-	-
Stomach	NE	0/4	-
Cervix	NE	-	-
Head and Neck	NE	2/7	-
Bladder	NE	-	-
Kidney	NE	0/10	-
Esophagus	NE	-	-
Brain	NE	-	-
Testicle	NE	0/2	-
Melanoma	NE	0/3	-
Sarcoma	NE	1/7	-
Uterus	NE	-	-

Table 4. 28. --Activity of 6-TG

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
None	++		
None	+		
Colon	-	1/35	3
Lung	NE	-	-
Breast	NE	-	-
Pancreas	NE	-	-
Ovary	NE	-	-
Prostate	NE	-	-
Stomach	NE	-	-
Cervix	NE	-	-
Head and Neck	NE	-	-
Bladder	NE	-	-
Kidney	NE	-	-
Esophagus	NE	-	-
Brain	NE	-	-
Testicle	NE	-	-
Melanoma	NE	-	-
Sarcoma	NE	-	-
Uterus	NE	-	-

Table 4. 29. --Activity of VBL

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Breast	++	19/95	20
Testicle	++	17/25	52
Head and Neck	+	10/35	29
Brain	+	21/48	44
Colon	-	4/64	5
Lung	-	17/239	7
Melanoma	-	11/71	15
Pancreas	NE	-	-
Ovary	NE	3/20	15
Prostate	NE	1/2	-
Stomach	NE	-	-
Cervix	NE	-	-
Bladder	NE	-	-
Esophagus	NE	-	-
Kidney	NE	1/12	-
Sarcoma	NE	-	-
Uterus	NE	-	-

Table 4. 30. --Activity of VCR

Tumor	Activity	No. of responses/ No. of evaluable patients	Response rate (%)
Breast	++	47/226	20
Sarcoma	++	25/53	47
Cervix	+	10/34	29
Brain	+	33/58	57
Lung	+	6/43	14
Colon	-	0/26	-
Ovary	-	0/17	-
Melanoma	-	6/52	12
Pancreas	NE	-	-
Prostate	NE	2/22	9
Stomach	NE	-	-
Head and Neck	NE	-	-
Bladder	NE	-	-
Kidney	NE	-	-
Esophagus	NE	-	-
Testicle	NE	4/7	-
Uterus	NE	-	-

TABLE 5.—Ranking of tumors on the basis of the percentage of drugs adequately evaluated

Rank	Tumor type	No. of drugs (of 30) adequately evaluated*	%
1	Colon	25	83
2	Breast	24	80
3	Melanoma	23	77
4	Lung	22	73
5	Head and neck	16	53
6	Sarcoma	11	37
7	Ovary	11	37
8	Cervix	10	33
9	Brain	8	27
10	Kidney	8	27
11	Stomach	8	27
12	Testicle	6	20
13	Prostate	5	17
14	Pancreas	3	10
15	Bladder	3	10
16	Esophagus	1	3
17	Uterus	0	—

*Includes only drugs rated −, +, or ++ in table 2.

TABLE 6.—Ranking of tumors by percentage of drugs showing definite activity

Rank	Tumor type	No. drugs with definite activity*/ No. drugs adequately evaluated	%
1	Testicle	4/6	66
2	Ovary	7/11	64
3	Brain	3/8	38
4	Sarcoma	4/11	36
5	Breast	8/24	33
6	Bladder	1/3	33
7	Pancreas	1/3	33
8	Lung	6/22	27
9	Stomach	2/8	25
10	Cervix	2/10	20
11	Head and neck	2/16	13
12	Colon	3/25	12
13	Melanoma	1/23	4
14	Kidney	0/8	—
15	Prostate	0/5	—
16	Esophagus	0/1	—
17	Uterus	0/0	—

*Includes only drugs rated ++ in table 2.

TABLE 7.—Ranking of tumors by percentage of drugs showing definite activity or evidence of activity

Rank	Tumor type	No. of drugs with evidence of activity or definite activity*/ No. drugs adequately evaluated	%
1	Cervix	10/10	100
2	Testicle	6/6	100
3	Bladder	3/3	100
4	Esophagus	1/1	100
5	Brain	7/8	88
6	Ovary	9/11	82
7	Head and neck	12/16	75
8	Stomach	6/8	75
9	Sarcoma	8/11	73
10	Pancreas	2/3	66
11	Lung	14/22	64
12	Prostate	3/5	60
13	Breast	13/24	54
14	Colon	7/25	28
15	Melanoma	6/23	26
16	Kidney	2/8	25
17	Uterus	0/0	—

*Includes only drugs rated + or ++ in table 2.

TABLE 8.—Ranking of drugs by percentage of tumors in which they have been adequately evaluated

Rank	Drug	No. of tumors (of 17) adequately evaluated*	%
1	5-FU	12	70
2	ADR	12	70
3	MCCNU	11	65
4	HXM	9	53
5	CCNU	9	53
6	CYC	9	53
7	CHL	8	47
8	VCR	8	47
9	BLM	8	47
10	MTX	8	47
11	MTC	8	47
12	HN2	8	47
13	VBL	7	41
14	BCNU	7	41
15	DTIC	7	41
16	DBD	7	41
17	MLP	7	41
18	MTH	7	41
19	HYD	6	35
20	ACT	5	30
21	PCB	5	30
22	6-MP	5	30
23	Ara-C	4	24
24	STZ	2	12
25	BUS	2	12
26	5-AZC	2	12
27	6-TG	1	9
28	L-ASP	0	—
29	DNR	0	—
30	DBM	0	—

*Includes only drugs rated −, +, or ++ in table 2.

TABLE 9.—Ranking of drugs based on the number of tumors against which they are definitely active

Rank	Drug	No. tumors showing definite response*/ No. tumors adequately evaluated	%
1	CYC	5/9	63
2	5-FU	6/12	50
3	ADR	5/12	42
4	ACT	2/5	40
5	MTX	3/8	38
6	MTC	3/8	38
7	MLP	2/7	29
8	VBL	2/7	29
9	VCR	2/8	25
10	BLM	2/8	25
11	HN2	2/8	25
12	CCNU	2/9	22
13	HXM	2/9	22
14	MCCNU	2/11	18
15	MTH	1/7	14
16	BCNU	1/7	14
17	DTIC	1/7	14
18	CHL	1/8	13
19	DBD	0/7	—
20	PCB	0/5	—
21	6-MP	0/5	—
22	HYD	0/6	—
23	Ara-C	0/4	—
24	BUS	0/2	—
25	STZ	0/2	—
26	5-AZC	0/2	—
27	6-TG	0/1	—
28	DNR	0/0	—
29	L-ASP	0/0	—
30	DBM	0/0	—

*Includes only tumors ranked ++ in table 2.

TABLE 10.—Ranking of drugs based on the number of tumors against which they are definitely active or show evidence of activity

Rank	Drug	No. tumors showing evidence of response or definite response*/ No. tumors adequately evaluated	%
1	CYC	8/9	89
2	MTX	7/8	88
3	MTC	7/8	88
4	BCNU	6/7	86
5	DBD	6/7	86
6	5-FU	9/12	75
7	ADR	9/12	75
8	CHL	6/8	75
9	HXM	6/9	66
10	VCR	5/8	63
11	BLM	5/8	63
12	HN2	5/8	63
13	ACT	3/5	60
14	MLP	4/7	57
15	VBL	4/7	57
16	MCCNU	6/11	55
17	HYD	3/6	50
18	CCNU	4/9	44
19	PCB	2/5	40
20	DTIC	2/7	29
21	6-MP	1/5	20
22	MTH	1/7	14
23	Ara-C	0/4	—
24	BUS	0/2	—
25	STZ	0/2	—
26	5-AZC	0/2	—
27	6-TG	0/1	—
28	DNR	0/0	—
29	L-ASP	0/0	—
30	DBM	0/0	—

*Includes only tumors rated + or ++ in table 2.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
L-Asparaginase: NSC-109229; CAS reg. No. 9015-68-3
5-Azacytidine: NSC-102816; CAS reg. No. 320-67-2; *s*-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-
BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-
Bleomycin: NSC-125066
Busulfan: NSC-750; CAS reg. No. 55-98-1; 1,4-butanediol, dimethanesulfonate
CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
Chlorambucil: NSC-3088; CAS reg. No. 305-03-3; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6
Dibromodulcitol: NSC-104800; CAS reg. No. 10318-26-0; galactitol, 1,6-dibromo-1,6-dideoxy-
Dibromomannitol: NSC-94100; CAS reg. No. 488-41-5; 1,6-dibromo-1,6-dideoxy-D-mannitol
DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8
Hexamethylmelamine: NSC-13875; CAS reg. No. 645-05-6
Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1
Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride
Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-
6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-
Mithramycin: NSC-24559; CAS reg. No. 1837d-89-7
Mitomycin C: NSC-26980; CAS reg. No. 50-07-7
Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride
Streptozotocin: NSC-85998
6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-
Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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ADDENDUM

Brain Cancer

5-Fluorouracil was negative in a controlled surgical adjuvant study with no effect on survival. (Edland et al, Am J Roentgenol Radium Ther Nucl Med 111:337, 1971) and hence its activity is considered "-."

Adriamycin

Further data on adriamycin may be found in this issue of *Cancer Chemotherapy Reports*. Additional data have led to adriamycin showing "+" activity in prostate cancer (10/52 = 19%) and "+" activity in uterine cancer (7/18 = 38%).

The above additions in data would cause minor changes in tables 5-10.

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